

TRADE SECRET

Unpublished Work

Copyright ©2010 E.I. du Pont de Nemours and Company

STUDY TITLE: H-28548: Early Life-Stage Toxicity to Rainbow Trout,
Oncorhynchus mykiss

TEST GUIDELINES: U.S. EPA, OPPTS 850.1400: Fish Early-Life Stage Toxicity
Test, *Ecological Effects Test Guidelines* (Public Draft, 1996)

OECD Guideline for Testing of Chemicals
Section 2 (Part 210) (1992)

AUTHOR: Robert A. Hoke, Ph.D.

STUDY COMPLETED ON: November 15, 2010

PERFORMING LABORATORY: E.I. du Pont de Nemours and Company
DuPont Haskell Global Centers
for Health & Environmental Sciences
P.O. Box 50
Newark, Delaware 19714
U.S.A.

LABORATORY PROJECT ID: DuPont-18405-217

WORK REQUEST NUMBER: 18405

SERVICE CODE NUMBER: 217

SPONSOR: E.I. du Pont de Nemours and Company
Wilmington, Delaware 19898
U.S.A.

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was conducted in compliance with U.S. EPA TSCA (40 CFR part 792) Good Laboratory Practice Standards, which are compatible with current OECD Good Laboratory Practices, except for the item documented below. The item listed does not impact the validity of the study.

1. Test substance solubility and stability in the dilution water were determined in a previous study.⁽¹⁾

Sponsor: E.I. du Pont de Nemours and Company
Wilmington, Delaware 19898
U.S.A.

Study Director: Robert A. Hoke 15 NOV 2010
Robert A. Hoke, Ph.D. Date
Principal Research Ecotoxicologist

Sponsor: _____
DuPont Representative Date

QUALITY ASSURANCE STATEMENT

Work Request Number: 18405
Service Code Number: 217

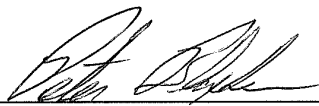
Key inspections for the above referenced study were completed by the Quality Assurance Unit of DuPont Haskell and the findings were submitted on the following dates:

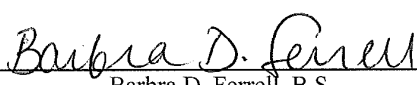
<i>Audit Dates</i>	<i>Date Reported to Study Director</i>	<i>Date Reported to Management</i>
<u>Protocol:</u> January 18, 2010	January 18, 2010	January 18, 2010
<u>Conduct:</u> April 14, 2010 July 28, 2010	April 14, 2010 July 28, 2010	April 14, 2010 July 28, 2010
<u>Report/Records:</u> October 15, 18-22, 25, 2010	October 25, 2010	October 26, 2010

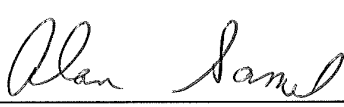
Reported by: Donna M. Johnston for A.P. 09 Nov. 2010
Antonio Pedulla Date
Quality Assurance Auditor

CERTIFICATION

We, the undersigned, declare that this report provides an accurate evaluation of data obtained from this study.

Analytical Evaluation by:  15-Nov-2010
Peter A. Bloxham, Ph.D.
Senior Research Chemist
Date

Statistical Analysis by:  09-Nov-2010
Barbra D. Ferrell, B.S.
Associate Scientist
Date

Reviewed by:  15-Nov-2010
Alan Samel, M.S.
Global Regulatory Ecotoxicologist
Date

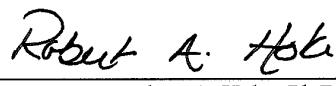
Issued by Study Director:  15 NOV 2010
Robert A. Hoke, Ph.D.
Principal Research Ecotoxicologist
Date

TABLE OF CONTENTS

	Page
GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT	2
QUALITY ASSURANCE STATEMENT	3
CERTIFICATION.....	4
TABLE OF CONTENTS	5
LIST OF TABLES	6
LIST OF FIGURES	7
LIST OF APPENDICES	7
STUDY INFORMATION	8
SUMMARY	9
INTRODUCTION.....	11
ANIMAL WELFARE ACT COMPLIANCE	11
MATERIALS AND METHODS	11
A. Test Guidelines	11
B. Test Substance	12
C. Dilution Water	12
D. Test Organism Culture.....	12
E. Test Methods.....	13
F. Sample Preparation and Chemical Analysis	15
1. Sample Collection and Treatment.....	15
2. Instrument and Conditions	16
3. Quantitation.....	16
G. Statistical Analysis.....	17
RESULTS	18
A. Analytical Report.....	18
1. Chromatographic Results	18
2. QC Sample Results	18
3. Test Solution Results.....	18
B. In-Life Report	18
C. Statistical Analysis Report.....	19
CONCLUSION	20
RECORDS AND SAMPLE STORAGE.....	20
REFERENCES.....	20
TABLES.....	22
FIGURES.....	113
APPENDIX.....	119

LIST OF TABLES

	Page
Table 1	Chemical Characteristics of Haskell Well Water.....23
Table 2	Measured Concentrations of H-28548 in Test Solutions24
Table 3	Water Chemistry of the Dilution Water Control and Highest Test Concentration with Surviving Organisms27
Table 4	Temperature (°C) of Test Solutions for the 90-Day Rainbow Trout, <i>Oncorhynchus mykiss</i> , Early Life-Stage Toxicity Test28
Table 5	pH of Test Solutions for the 90-Day Rainbow Trout, <i>Oncorhynchus mykiss</i> , Early Life-Stage Toxicity Test29
Table 6	Dissolved Oxygen Concentration (mg/L) of Test Solutions for the 90-Day Rainbow Trout, <i>Oncorhynchus mykiss</i> , Early Life-Stage Toxicity Test30
Table 7	Summary of Hatching, Survival, Abnormalities, and Swim-Up from Hatching to Thinning for the 90-Day Rainbow Trout, <i>Oncorhynchus mykiss</i> , Early Life-Stage Toxicity Test31
Table 8	Summary of Survival, Abnormalities, and Growth from Thinning to Test End for the 90-Day Rainbow Trout, <i>Oncorhynchus mykiss</i> , Early Life-Stage Toxicity Test33
Table 9	Dilution Water Control: Embryo Survival and Hatching Data for the 90-Day Rainbow Trout, <i>Oncorhynchus mykiss</i> , Early Life-Stage Toxicity Test34
Table 10	0.651 mg/L: Embryo Survival and Hatching Data for the 90-Day Rainbow Trout, <i>Oncorhynchus mykiss</i> , Early Life-Stage Toxicity Test41
Table 11	1.08 mg/L: Embryo Survival and Hatching Data for the 90-Day Rainbow Trout, <i>Oncorhynchus mykiss</i> , Early Life-Stage Toxicity Test48
Table 12	2.16 mg/L: Embryo Survival and Hatching Data for the 90-Day Rainbow Trout, <i>Oncorhynchus mykiss</i> , Early Life-Stage Toxicity Test55
Table 13	4.66 mg/L: Embryo Survival and Hatching Data for the 90-Day Rainbow Trout, <i>Oncorhynchus mykiss</i> , Early Life-Stage Toxicity Test62
Table 14	8.89 mg/L: Embryo Survival and Hatching Data for the 90-Day Rainbow Trout, <i>Oncorhynchus mykiss</i> , Early Life-Stage Toxicity Test69
Table 15	Dilution Water Control: Swim-Up Data for the 90-Day Rainbow Trout, <i>Oncorhynchus mykiss</i> , Early Life-Stage Toxicity Test76
Table 16	0.651 mg/L: Swim-Up Data for the 90-Day Rainbow Trout, <i>Oncorhynchus mykiss</i> , Early Life-Stage Toxicity Test78
Table 17	1.08 mg/L: Swim-Up Data for the 90-Day Rainbow Trout, <i>Oncorhynchus mykiss</i> , Early Life-Stage Toxicity Test80
Table 18	2.16 mg/L: Swim-Up Data for the 90-Day Rainbow Trout, <i>Oncorhynchus mykiss</i> , Early Life-Stage Toxicity Test82
Table 19	4.66 mg/L: Swim-Up Data for the 90-Day Rainbow Trout, <i>Oncorhynchus mykiss</i> , Early Life-Stage Toxicity Test84
Table 20	8.89 mg/L: Swim-Up Data for the 90-Day Rainbow Trout, <i>Oncorhynchus mykiss</i> , Early Life-Stage Toxicity Test86
Table 21	Dilution Water Control: Larval Mortality After Thinning for the 90-Day Rainbow Trout, <i>Oncorhynchus mykiss</i> , Early Life-Stage Toxicity Test88

Table 22	0.651 mg/L: Larval Mortality After Thinning for the 90-Day Rainbow Trout, <i>Oncorhynchus mykiss</i> , Early Life-Stage Toxicity Test	91
Table 23	1.08 mg/L: Larval Mortality After Thinning for the 90-Day Rainbow Trout, <i>Oncorhynchus mykiss</i> , Early Life-Stage Toxicity Test	94
Table 24	2.16 mg/L: Larval Mortality After Thinning for the 90-Day Rainbow Trout, <i>Oncorhynchus mykiss</i> , Early Life-Stage Toxicity Test	97
Table 25	4.66 mg/L: Larval Mortality After Thinning for the 90-Day Rainbow Trout, <i>Oncorhynchus mykiss</i> , Early Life-Stage Toxicity Test	100
Table 26	8.89 mg/L: Larval Mortality After Thinning for the 90-Day Rainbow Trout, <i>Oncorhynchus mykiss</i> , Early Life-Stage Toxicity Test	103
Table 27	Dilution Water Control: Fish Lengths and Weights at Test Conclusion for the 90-Day Rainbow Trout, <i>Oncorhynchus mykiss</i> , Early Life-Stage Toxicity Test	106
Table 28	0.651 mg/L: Fish Lengths and Weights at Test Conclusion for the 90-Day Rainbow Trout, <i>Oncorhynchus mykiss</i> , Early Life-Stage Toxicity Test	107
Table 29	1.08 mg/L: Fish Lengths and Weights at Test Conclusion for the 90-Day Rainbow Trout, <i>Oncorhynchus mykiss</i> , Early Life-Stage Toxicity Test	108
Table 30	2.16 mg/L: Fish Lengths and Weights at Test Conclusion for the 90-Day Rainbow Trout, <i>Oncorhynchus mykiss</i> , Early Life-Stage Toxicity Test	109
Table 31	4.66 mg/L: Fish Lengths and Weights at Test Conclusion for the 90-Day Rainbow Trout, <i>Oncorhynchus mykiss</i> , Early Life-Stage Toxicity Test	110
Table 32	8.89 mg/L: Fish Lengths and Weights at Test Conclusion for the 90-Day Rainbow Trout, <i>Oncorhynchus mykiss</i> , Early Life-Stage Toxicity Test	111
Table 33	90-day EC ₅₀ , NOEC, and LOEC Values for Rainbow Trout, <i>Oncorhynchus mykiss</i> , Based on Mean, Measured Concentrations of H-28548	112

LIST OF FIGURES

		Page
Figure 1	Photograph of an Embryo at Approximately 22 Hours Post-Fertilization	114
Figure 2	Representative Analytical Calibration Standard Curve for H-28548.....	115
Figure 3	Representative HPLC Chromatogram of a Calibration Standard Solution	116
Figure 4	Representative HPLC Chromatogram of a HWW Control Solution.....	117
Figure 5	Representative HPLC Chromatogram of a H-28548 Test Solution	118

LIST OF APPENDICES

	Page
Appendix A Certificate of Analysis	120

STUDY INFORMATION

Substance Tested:

- HFPO Dimer Acid Ammonium Salt
- 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propionic acid, ammonium salt
- 62037-80-3 (CAS Number)
- H-28548

Haskell Number: 28548

Composition: Proprietary

Purity: 84%

Physical Characteristics: Clear and colorless liquid

Stability: The test substance appeared to be stable under the conditions of the study; no evidence of instability was observed.

Study Initiated/Completed: January 15, 2010 / (see report cover page)

Experimental Start/Termination: April 9, 2010 / July 28, 2010

In-Life Initiated/Completed: April 9, 2010 / July 28, 2010

Notebook Number(s): E-114318-BB, E112725-BP

SUMMARY

GUIDELINES: The effect of H-28548 (purity 84%) on hatching, growth and survival of rainbow trout, *Oncorhynchus mykiss*, embryos, alevins, and fingerlings was assessed in an intermittent-flow, 90-day early life-stage toxicity test in accordance with the appropriate Good Laboratory Practice standards and test guidelines (U.S. EPA OPPTS 850.1400; OECD 210).

NOMINAL

TEST SUBSTANCE 0.63, 1.25, 2.50, 5.00, and 10.0 mg/L H-28548 (corrected for
CONCENTRATIONS: 84% purity), dilution water control

MEASURED

TEST SUBSTANCE 0.651, 1.08, 2.16, 4.66, and 8.89 mg/L H-28548, none detected
CONCENTRATIONS: in dilution water control (LOD 0.009 mg/L)

AGE OF TEST

ORGANISMS: Approximately 24 hours post-fertilization at test start

SOURCE OF Unfertilized eggs and sperm were obtained from Troutlodge

TEST ORGANISMS: Inc. (Sumner, Washington)

TEST DESIGN: A total of 80 embryos were exposed per concentration (20 embryos per embryo cup, 2 cups per replicate, 2 replicates per concentration) at test start. On day 46, after swim-up had begun in the dilution water control, the fingerlings were thinned to a total of 30 fish per concentration (15 fish per replicate, 2 replicates per concentration). Analytical verification of H-28548 concentrations was made on test solutions sampled on day -1, day 0, once weekly, and on day 90 at test end. Additional samples were submitted for analysis as warranted.

Test solutions were supplied to each replicate test chamber by an intermittent-flow, proportional diluter system, at a turnover rate (based on one diluter cycle per 22-24 minutes) of approximately 30 L per replicate per day, which is equivalent to five, 6-L test solution volumes per day. Embryos and alevins were held in relative darkness until day 30 and then held under a photoperiod of 16 hours light and 8 hours darkness (which included 30 minutes of transitional light between light and dark intervals) for the remainder of the study. After thinning, the fingerlings were fed newly-hatched brine shrimp (days 46-89) 3 times daily on weekdays and

twice daily on weekends and holidays. Approximately 7-10 days after transfer, the newly-hatched brine shrimp were supplemented once daily with trout chow (days 53-89). Test solutions were maintained between 11.8 and 12.9°C (mean 12.5°C). Observations were made for assessment of the number of dead eggs, first and last day of hatching, first day of swim-up, survival and abnormalities from hatching to thinning, and survival and abnormalities from thinning to test end. Standard length and blotted wet weight of surviving fingerlings were determined at test end.

CONCLUSION: The 90-day EC₅₀ values based on mean, measured concentrations and all measured parameters were greater than 8.89 mg/L.

The 90-day NOEC and LOEC values based on mean, measured concentrations and last day of hatching were determined to be 1.08 mg/L and 2.16 mg/L, respectively. The 90-day NOEC and LOEC values based on mean, measured concentrations and all other endpoints were determined to be 8.89 mg/L, the highest tested concentration, and greater than 8.89 mg/L, respectively. The 90-day EC₅₀ values based on mean, measured concentrations and all endpoints were greater than 8.89 mg/L.

Evaluation of the actual data for mean last day of hatching indicated that it ranged from 24 days in the control to 23 days in the highest three test concentrations. Based on the lack of any other significant effects on the endpoints evaluated at any concentration less than 8.89 mg/L H-28548, the slight decrease in last day of hatching does not appear to be a significant biological effect and the overall study NOEC and LOEC are therefore 8.89 and greater than 8.89 mg/L H-28548.

STUDY

COMPLETION: (see report cover page)

INTRODUCTION

The purpose of this study was to assess the early life-stage toxicity of H-28548 to embryos, alevins, and fingerlings of rainbow trout, *Oncorhynchus mykiss*, in an intermittent renewal, 90-day test.

ANIMAL WELFARE ACT COMPLIANCE

This study complied with all applicable sections of the Final Rules of the Animal Welfare Act regulations (9 CFR) and the Guidelines from the Guide for the Care and Use of Laboratory Animals (NRC 1996). All studies conducted by or for DuPont Haskell adhere to the following principles:

- The sponsor and/or the study director ensures that the study described in this report does not unnecessarily duplicate previous experiments, and is in compliance with the DuPont Policy on Animal Testing.
- Whenever possible, procedures used in this study have been designed to implement a reduction, replacement, and/or refinement in the use of animals in an effort to avoid or minimize discomfort, distress or pain to animals. All methods are described in this study report or in written laboratory standard operating procedures.
- Methods of euthanasia used during this study were in conformance with the above referenced regulation and the recommendations of the American Veterinary Medical Association (AVMA), 2007 Guidelines on Euthanasia.
- The procedures in this protocol have been reviewed by the Haskell Animal Welfare Committee and comply with acceptable standards for animal welfare and humane care.
- DuPont Haskell is accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC) International.

MATERIALS AND METHODS

A. Test Guidelines

The study design complied with the following test guidelines:

- U.S. EPA, OPPTS 850.1400: Fish Early-Life Stage Toxicity Test, *Ecological Effects Test Guidelines* (Public Draft, 1996)⁽¹⁾.
- OECD, Section 2 (Part 210): Fish, Early-Life Stage Toxicity Test, *Guideline for Testing of Chemicals* (1992).⁽²⁾

B. Test Substance

The test substance, H-28548, was supplied by the sponsor as a liquid.

H-28548 contains 84% active ingredient by analysis (Appendix A).

The solubility of H-28548 in Haskell well water (HWW) was determined in a previous study to be 218 mg/L, after 25 hours of stirring, at approximately 10°C⁽³⁾. The stability of H-28548 in HWW was demonstrated for up to 96 hours at 4°C and 20°C⁽³⁾.

A stock solution was prepared at a concentration of approximately 10 mg/L, adjusted for test substance purity of 84%, on a daily basis in HWW. The stock solution (140 L total volume) was prepared by direct addition of the test substance into a large stainless steel mixing tank contained within the test system waterbath. After the stock solution volume was brought to 140 L with HWW, the stock solution was stirred for 28-57 minutes and then pumped into a large stainless steel delivery tank also contained within the test system waterbath. Throughout the test, the stock solutions and test solutions were clear and colorless with no visible precipitate.

C. Dilution Water

Dilution water originated from the Haskell well which is 480-feet deep and is cased and sealed to bedrock. The hardness of the HWW is adjusted to approximately 100-140 mg/L as CaCO₃ by the flow-proportioned addition of CaCl₂. The HWW is then aerated, passed through a green sand filter to remove iron, and filtered through 50-, 10-, and 0.45-µm cartridge filters to remove particulates. The water is heated or chilled as appropriate and distributed through aged polyvinyl chloride piping. The dilution water is analyzed at least once yearly for major anions and cations, metals, total organochlorine and organophosphate pesticides, and polychlorinated biphenyls (Table 1). The dilution water meets OECD⁽²⁾ and ASTM⁽⁵⁾ criteria and specifications.

D. Test Organism Culture

Rainbow trout, *Oncorhynchus mykiss*, unfertilized eggs and sperm were purchased from Troutlodge, Inc., Sumner, Washington. Eggs and sperm were received in separate plastic bags which were kept on ice (approximately 1.0 – 2.0°C) during shipping. Eggs and sperm were allowed to equilibrate to approximately 11.9°C in a waterbath. Approximately 200 mL of the buffer solution containing glycine, TRIS, and sodium chloride was added to the bag containing the eggs. The purpose of the buffer was to remove any ovarian fluid that could impede fertilization. The bag was placed back into the waterbath for approximately 20 to 30 minutes. The bag of eggs was taken out of the waterbath and the buffer solution was removed. The bag of sperm was removed from the waterbath and its contents were added to the bag of eggs. The bag was gently rocked back and forth to evenly distribute the sperm. Buffer solution (50 mL) was also added to the bag to help activate the sperm. The bag was again gently rocked back and forth and returned to the waterbath for about 15 minutes. The embryos were removed from the waterbath and the buffer solution and sperm poured off of the embryos. The embryos were then rinsed with cold HWW to remove residual sperm and other debris before being transferred to two stainless steel tanks, each containing 10 L of aerated HWW, in a waterbath (approximately 11.9°C) under darkness. Approximately 24 hours later, embryos were randomly placed into

embryo cups to begin the test. The embryonic stage at the beginning of exposure was verified by selecting a representative sample of fertilized eggs, photographing, and comparing with literature sources.⁽⁴⁾ Embryos and larvae were identified by labels on aquaria and test chambers.

E. Test Methods

Five test substance concentrations and a water control were used in this study. Nominal H-28548 active substance concentrations (corrected for 84% purity) were 0.63, 1.25, 2.50, 5.00, and 10.0 mg/L.

Test chambers (2 replicate chambers per concentration) were 13-L stainless steel aquaria (30 [length] × 14.5 [width] × 30 [height] cm), each holding approximately 6 liters of solution (15-cm liquid depth) and fitted with a screen mesh-covered overflow pipe. A recirculating waterbath was used to maintain temperature in the test chambers at $12 \pm 2^{\circ}\text{C}$. Test chambers were positioned in the waterbath using random numbers.

Test solutions were supplied to the test chambers by an intermittent-flow, proportional diluter system. Diluter surfaces exposed to test solutions consisted of glass, Tygon[®], Teflon[®], or stainless steel. A stock solution was prepared and mixed in a stainless steel stock tank housed in the waterbath. After mixing, the stock solution was transferred to a second stock tank or diluter supply tank housed in the waterbath.

The diluter was set up to complete one cycle approximately every 22-24 minutes. During a diluter cycle, the stock solution was pumped from the diluter supply tank to a sheer pump mixing chamber. The sheer pump operated at a high velocity to provide additional mixing of the stock solution. After the timed cycle for the sheer pump, a solenoid opened and transferred the stock solution into pre-calibrated head chambers that delivered appropriate volumes of stock solution. Once the head chambers were filled, all the solenoids opened to release the stock solution volumes and Haskell well water into the appropriate test solution mixing chambers. The stock solution also served as the high test substance concentration solution. The test solutions in each mixing chamber (total volume ~ 1000 mL) were then equally split and delivered into the corresponding replicate test chambers. The turnover rate based on one diluter cycle per 22-24 minutes was approximately 30 L per replicate per day, which is equivalent to five, 6-L test solution volumes per day.

Two 212-mL glass embryo cups (5.5-cm diameter) with screen mesh bottoms attached with silicone adhesive were suspended in each replicate. Twenty embryos were placed into each embryo cup (total of 40 embryos per replicate test chamber and 80 embryos per test substance concentration or control). Embryo cups were assigned to each replicate and embryos were assigned to embryo cups using random numbers. Embryo cups oscillated at approximately 4 rpm (2 to 8-cm water depth in embryo cup). Embryo cups were inspected daily and unfertilized eggs and damaged or fungus-infected embryos were removed. The criterion for embryo death was opaque appearance.

The surviving alevins and fingerlings were thinned to 15 per replicate using random numbers and released into the appropriate test chamber replicate on day 46 after swim-up had begun in the dilution water control. Fish that swim-up are those that occupy and are able to maintain their

position in the top half of the water column and in which the yolk sac has mostly been resorbed. On day 46, feeding was initiated. The fingerlings were fed newly-hatched brine shrimp (*Artemia* sp., *ad libitum*, (San Francisco Bay Brand, Newark, California)) 3 times daily on weekdays and twice daily on weekends and holidays. On day 53 through day 89, one of the daily feedings with brine shrimp was supplemented once daily with trout chow (Aqua Max[®] Starter Fingerling 300 5D03 (PMI[®] Nutrition International, LLC)). The fingerlings were last fed on day 89 (morning, brine shrimp and trout chow), approximately 24 hours prior to test conclusion on the following day. The brine shrimp eggs and trout chow are typically analyzed prior to use of a new lot of food to demonstrate that any contaminants present are at, or below, acceptable concentrations. The concentration of total organic chlorine should not exceed 0.15 µg/g wet weight or the total organochlorine pesticides plus PCBs should not exceed 0.3 µg/g wet weight. Uneaten food and/or debris were removed as needed. The number of live alevins and fingerlings and the occurrence of any abnormal behavior or deformities was determined daily by actual count and recorded in the study records. Dead alevins and fingerlings were enumerated and removed upon observation. Criteria for alevin and fingerling death were the absence of opercular movement and lack of reaction to gentle prodding. Fish were humanely sacrificed using MS-222 at test end, the standard length of all surviving fish measured to the nearest 0.1 cm, the corresponding wet weight for each fish determined to the nearest 0.01 mg, and these data were entered into the study records.

Diluter operation was checked daily. Test solution concentrations were verified by chemical analysis prior to beginning the test (day -1), at test start (day 0), approximately weekly thereafter, and at test end. Before thinning, water samples were taken from a point between the embryo cups at the center, mid-depth point from the larger replicate test chambers (one sample per replicate, 2 samples per concentration plus an equal number of backup samples). After thinning, samples were taken from the same location in each test chamber (one sample per replicate, 2 samples per concentration plus an equal number of backup samples). Loading in the "A" replicate of the dilution water control at the end of the test was 0.785 g fish per liter passing through the test chamber in 24 hours, based on a daily turnover rate of 30 L or approximately five, 6-L test solution volumes. Test solutions were unaerated through day 80, however, test solutions were aerated from day 81 through the end of the study. Aeration had no effect on test concentrations based on the analytical verification of test concentrations. Test solution temperatures were maintained between 11.8 and 12.9°C (mean = 12.5°C). The diluter was wrapped in black plastic to keep the embryos and alevins in relative darkness until the completion of hatching. The black plastic was removed from day 30 onwards. A photoperiod of 16 hours light (approximately 237 - 371 Lux) and 8 hours darkness, which included 30 minutes of transitional light (approximately 4 - 159 Lux) between the light and dark intervals, was used throughout the remainder of the study.

Dissolved oxygen, pH, and temperature were measured in both replicates of the dilution water control and all test concentrations at the beginning of the test before embryos were added, weekly, and at test end. A continuously-recording thermometer was used to check temperature variations in the test system waterbath. Total alkalinity, EDTA hardness, and conductivity were measured in the "A" replicate of the dilution water control and the highest test substance concentration with surviving test organisms at the beginning of the test, weekly, and at test end.

F. Sample Preparation and Chemical Analysis

1. Sample Collection and Treatment

One sample plus a back-up sample of each test solution was received from each replicate test chamber at all test concentrations including the Haskell well water (HWW) control on days -1, 0, 6, and weekly thereafter until the end of the definitive study (day 90). Samples from day -1 were analyzed to confirm proper functioning of the flow-through delivery system and were not used for calculation of mean, measured concentrations. During each sampling event for test solutions, samples were also collected of effluent from the carbon filter treatment system. Additional effluent samples were collected as necessary to evaluate performance of the carbon filter system. Samples and back-up samples were stored refrigerated upon receipt and when not in use.

The samples with nominal test concentrations (corrected for 84% purity) of 0.63, 1.25, 2.50, 5.00, and 10.00 mg/L were diluted 20× with HPLC grade water before analysis.

Quality control (QC) samples were prepared by adding 20.0 µL of an analytical standard stock solution and 980 µL of HWW control to an HPLC vial. All QC samples were diluted 20× in HPLC grade water prior to analysis.

Concentrations of H-28548 active substance were measured by high performance liquid chromatography with detection by mass spectrometry (LC/MS) within 1 day of sample receipt. All samples were stored refrigerated when not in use.

The original study was initiated on April 2, 2010 (day -6) and terminated on April 22, 2010 (day 13). Study samples were received and analyzed during this period but the data was not used for the final definitive study. No data from these samples is included in this report.

Primary samples received on day 62 of the study were re-prepared and re-analyzed due to contamination of the dilution solvent during the initial preparation of standards and samples for analysis. Data from the initial analysis was not used for the study and are not included in this report.

2. Instrument and Conditions

HPLC Instrument: Agilent Model 1100
MS Instrument: Quattro ZQ, Micromass
Software: MassLynx and QuanLynx v. 4.0

LC Parameters:

Column: Zorbax SB-C8, 100 × 2.1 mm, 3.5 µm particle size
Mobile Phase: A: HPLC grade water, 20 mM ammonium acetate
B: HPLC grade methanol, 20 mM ammonium acetate
Flow Rate: 0.400 mL/min
Mobile Phase Composition: Isocratic: A: 50% B: 50%
Column Temperature: 35°C
Autosampler Temperature: 15°C
Injection Volume: 10.0 µL

MS Parameters:

Capillary Voltage: 3.20 kV
Cone Voltage: 9.00 V
Source Temperature: 120°C
Desolvation Temperature: 300°C
Cone Gas Flow: 50 L/Hr
Desolvation Gas Flow: 411 L/Hr
Ionization Mode: Electrospray, Negative Ion

Data Acquisition Function: SIR of Mass 329
0.0 to 5.0 minutes
Dwell 0.20 seconds

3. Quantitation

A primary stock solution of the reference standard, H-28548 (purity 84%), was made by dissolving the standard in HPLC grade water. A secondary stock solution was made by dissolving an aliquot of the primary stock solution in HPLC grade water. On each day of analysis an aliquot of the secondary stock solution was diluted with HPLC grade water to prepare calibration standards with concentrations that bracketed the concentrations of the test solutions. Duplicate injections of test and calibration standard solutions were made and the peak areas were determined electronically.

The calibration standard curve was generated by regression analysis using the chromatographic peak areas of the calibration standard solutions. Data for test solutions were compared to the calibration standard curve to determine concentrations of H-28548. The limit of detection (LOD) was determined by calculating the average noise level in chromatograms of the HWW control solution samples and comparing them to the signal of a calibration standard of known concentration. Two chromatograms were examined for noise-related peaks near the retention time of the analyte. The LOD was calculated as 3 times the concentration equivalent of the mean

noise level. The limit of quantitation (LOQ) was established as the lowest calibration standard concentration.

G. Statistical Analysis

The NOEC for each response was determined through the use of trend tests (Cochran-Armitage trend test or the Jonckheere trend test) wherever appropriate. These trend tests assume monotonicity in the concentration-response. Parametric, or if appropriate, non-parametric tests, were used to determine if there was significant departure from monotonicity. For non-monotone data, other multiple comparison methods were used.

Hatching, survival and larval abnormality data were evaluated by the Cochran-Armitage test used in a step -down manner with equally-spaced concentration scores. If there was significant departure from monotonicity, then Fisher's exact test was used with a Bonferonni-Holm correction.

Continuous responses (length, weight, first and last day of hatching, first day of swimup) were evaluated for normality using the Shapiro-Wilk test while homogeneity of variance was assessed by Levene's test. Data found to be both normal and homogenous were evaluated in the context of ANOVA. Monotonic concentration-response data were evaluated using the Jonckheere-Terpstra trend test. Non-monotonic concentration-response data were assessed using the Dunnett method if the data were normally distributed and homogeneous; using the Tamhane-Dunnett (or T3) method if the data were normally distributed but heterogeneous. If factors in addition to concentration must be taken into account, such as the number of replicate test chambers in each test concentration at test start or test end, then the Jonckheere test was not appropriate and multi-factor ANOVA was used. Non-normal data were analyzed by alternative methods. If possible, a transformation was found to obtain normality. Otherwise, a Kruskal-Wallis test replaced the ANOVA, Dunn's test replaced Dunnett's, and the Jonckheere-Terpstra test was still applicable, except as noted. Outliers were determined by the Tukey outlier rule and, if present, their effect on the conclusions was determined.

If massive ties were present in a continuous response, exact permutation methods were used for data analysis. An exact version of the Kruskal-Wallis test was used, followed by exact versions of Jonckheere-Terpstra or separate Mann-Whitney (or, equivalently, Wilcoxon) comparisons of each treatment group to the dilution water control, using a Bonferonni correction.

Wherever possible, determination of the LC₅₀ and EC₅₀ was done by probit analysis for all frequency responses, adjusting for control mortality and using the mean measured concentration or its logarithmic transform, as appropriate. Where probit analysis failed, a moving-average angle or binary estimation procedure was used. Fieller's method (Inverse regression) was used to estimate the LC₅₀ for length and other continuous responses, if appropriate.

The No Observable Effect Concentration (NOEC) was defined as the highest measured concentration at or below which no statistically significant effect was observed. The Lowest Observed Effect Concentration (LOEC) was the lowest measured concentration at which a statistically significant effect occurred. The maximum acceptable toxicant concentration (MATC) was defined as the geometric mean of the NOEC and LOEC.

RESULTS

A. Analytical Report

1. Chromatographic Results

H-28548 eluted as a well-resolved chromatographic peak with a retention time of approximately 3.5 minutes. A typical calibration standard curve is shown in Figure 2. Representative chromatograms of a calibration standard solution, a HWW control solution sample, and a test solution sample are presented in Figures 3 to 5, respectively.

The LOD and LOQ were determined to be 0.009 mg/L and 0.100 mg/L, respectively, after application of a 20× dilution factor. For carbon filter effluent samples, which were analyzed without dilution, this corresponds to an LOD and LOQ of 0.0005 mg/L and 0.00500 mg/L, respectively.

2. QC Sample Results

The calculated recovery of the QC samples ranged from 93% to 109% with a mean recovery of 104%. These data confirm accurate quantitation by the analytical method during the study.

3. Test Solution Results

Mean, measured values of H-28548 ranged from 86% to 103% of the targeted nominal test concentrations corrected for purity (Table 2). All measured values of H-28548 were within 1.5× of the lowest value for all samples within a concentration. These data indicate that H-28548 concentrations were maintained at acceptable levels throughout the definitive test.

Control solutions contained no detectable concentrations of H-28548 (Table 2).

B. In-Life Report

Selection of test concentrations for the 90-day trout early-life stage study were based on a previous study.⁽¹⁰⁾ An initial definitive study was started on 9 April 2010 and terminated on 22 April 2010 due to poor fertilization success. The final definitive study was initiated on 29 April 2010.

Nominal H-28548 concentrations selected for the 90-day trout early life-stage study were 0.63, 1.25, 2.50, 5.00, and 10.0 mg/L. A dilution water control was used in this study. The corresponding mean, measured concentrations of H-28548 were 0.651, 1.08, 2.16, 4.66, and 8.89 mg/L. The dilution water control solutions showed no detectable levels of H-28548.

Dilution water quality was acceptable based on OECD⁽²⁾ and ASTM⁽⁵⁾ dilution water criteria. Based on the most recent semi-annual dilution water analysis (Table 1), contaminant concentrations were below concentrations that could be expected to affect the integrity of a study. All chemical and physical parameters in the early life-stage study were within expected ranges (Tables 3 to 6). Temperature and pH of the test substance and dilution water control

solutions ranged from 11.8 to 12.9°C (mean 12.5°C) and 7.4 to 8.3, respectively. Dissolved oxygen concentrations ranged between 5.8 and 10.3 mg/L (51 and 91% of air saturation based on an air saturation value of 11.3 mg/L at 12°C) over the duration of the study. Analyses of brine shrimp and trout chow indicated that total organochloride pesticides and PCBs were below concentrations that would be expected to affect the scientific integrity of the study.

A summary of hatching, swim-up, survival, and abnormalities from hatching to thinning is presented in Table 7. A summary of survival and abnormalities from thinning to test end, and mean length and wet weight (blotted dry) values at test end is presented in Table 8. Biological observations are presented in Tables 9 to 32. A photograph of representative fertilized eggs used to start the study is presented in Figure 1.

C. Statistical Analysis Report

Analyses are reported based on mean, measured H-28548 concentrations and were conducted using SAS Version 8.2.⁽⁶⁾ All statistical tests⁽⁷⁾ were calculated at a significance level of $p = 0.05$. A summary of the 90-day EC_{50} , 95% confidence intervals, LOEC, and NOEC values is presented in Table 33.

Data for first day of swim-up, first and last day of hatch are discrete and inherently non-normal, so non-parametric analyses were done for these three variables independent of any formal test of normality or variance homogeneity. Therefore, a non-parametric analysis was performed (Kruskal-Wallis test) followed by Dunn's test to compare test concentrations to the control to determine the NOEC and LOEC values. A non-linear trend was detected in the data for first day of swimup, so the Jonckheere-Terpstra test was not done.

No outliers were found in the data for first day of hatch. Outliers were found in the data for last day of hatch. However, upon further examination, outliers were not omitted from the data set. Ties, as determined by exact permutation data analysis methods, were present in the data for first day of hatch and last day of hatch, and exact permutation data analysis methods were recommended. Therefore, an exact permutation non-parametric analysis was performed (Kruskal-Wallis test) and an exact permutation tie-corrected Jonckheere-Terpstra test was used to determine the NOEC and LOEC values.

The data for fish length and weight were determined to be non-normally distributed. Outliers were found in the data for fish length and weight. However, upon further examination, outliers were not omitted from the data set. Ties, as determined by exact permutation data analysis methods, were present in the data for fish length and weight, but exact permutation data analysis methods were not required. Therefore, a non-parametric analysis was performed (Kruskal-Wallis test) and the Jonckheere-Terpstra test was used to determine the NOEC and LOEC values.

The data for egg survival, egg hatching, larvae survival, larval abnormalities, fish survival, and fish abnormalities are quantal data and are thus analyzed with the Cochran-Armitage test to determine NOEC and LOEC values and probit analysis to estimate EC_{50} values. Where there is significant non-monotonicity in the concentration-response, Fisher's Exact test with a Bonferroni-Holm adjustment to the p-values is used instead of the Cochran-Armitage test. These

tests are 1-sided against an increasing alternative where the response is expressed as egg or larvae or fish mortality or abnormality, or failure to hatch. Similarly, probit analyses are done on the same responses so that an increasing concentration-response is modeled. This prevents problems with probit modeling arising when there is background (or control) incidence.

The 90-day EC₅₀ values for all endpoints evaluated were greater than 8.89 mg/L H-28548. The NOEC values for all endpoints evaluated, except last day of hatching, were 8.89 mg/L H-28548 and the corresponding LOEC values were greater than 8.89 mg/L H-28548. The NOEC and LOEC values for last day of hatching were 1.08 and 2.16 mg/L H-28548, respectively.

Evaluation of the actual data for mean last day of hatching indicated that it ranged from 24 days in the control to 23 days in the highest three test concentrations. Based on the lack of any other significant effects on the endpoints evaluated at any concentration less than 8.89 mg/L H-28548, the slight decrease in last day of hatching does not appear to be a significant biological effect and the overall study NOEC and LOEC are therefore 8.89 and greater than 8.89 mg/L H-28548.

CONCLUSION

The 90-day EC₅₀ values based on mean, measured concentrations and all endpoints were greater than 8.89 mg/L. The 90-day NOEC and LOEC values based on mean, measured concentrations and the lack of significant biological effects at all test concentrations were determined to be 8.89 mg/L, the highest tested concentration, and greater than 8.89 mg/L, respectively.

RECORDS AND SAMPLE STORAGE

Specimens (if applicable), raw data, the protocol, amendments (if any), and the final report will be retained at DuPont Haskell, Newark, Delaware, Iron Mountain Records Management, Wilmington, Delaware, or Quality Associates Incorporated, Fulton, Maryland.

REFERENCES

1. U.S. EPA (Public Draft, 1996). Fish Early-Life Stage Toxicity Test. *Ecological Effects Test Guidelines*, OPPTS 850.1400.
2. OECD (1992). Fish, Early-Life Stage Toxicity Test. *Guideline for Testing of Chemicals*, Section 2 (Part 210).
3. DuPont Haskell (2007). Solubility Verification and Stability of H-28072 in Haskell Laboratory Well Water. Unpublished report, DuPont-22828.
4. Leitritz, E. and Lewis, R.C. (1976). Trout and salmon culture (hatchery methods), California Department of Fish and Game. *Fish Bulletin* 164, 197.

5. American Society for Testing and Materials (ASTM) (1992). Standard Guide for Conducting Early Life-Stage Toxicity Tests with Fishes. E1241-92. *Annual Book of ASTM Standards*, Vol. 11.04.
6. SAS Software Version 8.2 (1999). SAS Institute, Cary, North Carolina.
7. Organisation for Economic Co-Operation and Development (OECD). OECD series on testing and assessment. Number 54. Current approaches in the statistical analysis of ecotoxicity data: A guidance to application. 09-May-2006.
8. Tamhane, A.C., Dunnett, C.W., Green, J.W., and Wetherington, J.D. (2001). Multiple test procedures for identifying maximum safe dose. *J. Amer. Statist. Assoc.* 96, 835-843.
9. Stephan, C.E. (1972); Methods for calculating an LC₅₀, ASTM, Aquatic Toxicology and Hazard Assessment, First Annual Symposium on Aquatic Toxicology, F.L. Mayer and J.L. Hammerlink, eds., pages 65-84.
10. DuPont Haskell (2008). H-28072: Static, Acute, 96-Hour Limit Test with Rainbow Trout, *Oncorhynchus mykiss*. Unpublished report, DuPont-22830 Revision 1.

TABLES

Table 1
Chemical Characteristics of Haskell Well Water^a

Parameter	MDL ^b	Analytical Value	Parameter	MDL ^b	Analytical Value
BOD, mg/L	1.4	ND ^c	Lead, mg/L	0.0069	ND
COD, mg/L	12.8	ND	Magnesium, mg/L	0.0172	6.48
DOC, mg/L	0.50	ND	Manganese, mg/L	0.00084	ND
TOC, mg/L	0.50	ND	MBAS/LAS, mg/L	0.040	ND
Total Kjeldahl N, mg/L	0.50	ND	Mercury, mg/L	0.000056	ND
Ammonia N, mg/L	0.05	ND	Nickel, mg/L	0.0018	ND
Turbidity, NTU	0.09	0.15 J ^e	Nitrite, mg/L	0.015	ND
Phenolics, mg/L	0.015	ND	Nitrate, mg/L	0.04	ND
Color, apparent Co/Pt ^d	5.0	ND	Ortho-phosphate, mg/L	0.03	0.044 J
Solids			Potassium, mg/L	0.239	3.58
total suspended, mg/L	1.5	ND	Selenium, mg/L	0.00099	ND
Aluminum, mg/L	0.0802	ND	Silver, mg/L	0.000080	ND
Antimony, mg/L	0.0097	ND	Sodium, mg/L	0.433	9.45
Arsenic, mg/L	0.00095	ND	Sulfate, mg/L	0.3	2.8
Beryllium, mg/L	0.0014	ND	Sulfide, mg/L	0.054	ND
Boron, mg/L	0.0094	0.0110 J	Zinc, mg/L	0.0081	0.142 ^g
Bromide, mg/L	0.4	0.88	Ca/Mg	NA ^f	6.78
Cadmium, mg/L	0.00020	ND	Na/K	NA ^f	2.64
Calcium, mg/L	0.0702	43.9	Volatile priority		
Chloride, mg/L	4.0	51.2	pollutants, µg/L	0.5-40	ND
Chlorine, residual, mg/L	0.04	ND	Acid extractable		
Chromium, mg/L	0.00060	ND	priority pollutants, µg/L	1-20	ND
Cobalt, mg/L	0.0021	ND	Base/neutral		
Copper, mg/L	0.00038	ND	priority pollutants, µg/L	1-20	ND
Cyanide, mg/L	0.005	ND	Pesticides/PCBs, µg/L	0.0026-0.95	ND
Iron, mg/L	0.0522	ND	Organophosphate		
Fluoride, mg/L	0.080	0.25	pesticides, µg/L	0.47-1.9	ND

^a Sample analyses performed at Lancaster Laboratories, Lancaster, Pennsylvania, date of sample collection 21 December 2009 unless indicated otherwise,

^b MDL = method detection limit, ^c ND indicates not detected at the MDL, ^d Units based on cobalt/platinum reference, ^e A "J" follows analytical values which were greater than the MDL but less than the limit of quantitation, ^f NA = not applicable, ^g Zinc sample collected on 05 February 2010 in order to confirm December 2009 results.

Table 2
Measured Concentrations of H-28548 in Test Solutions

Nominal H-28548 Concentration, mg/L (corrected for purity)	Measured H-28548 Concentration (mg/L)							
	Day -1 ^a	Day 0	Day 6	Day 13	Day 20	Day 27	Day 34	Day 41
HWW Control A ^b HWW Control B	ND ^c	ND	ND	ND	ND	ND	ND	ND
	ND	ND	ND	ND	ND	ND	ND	ND
0.63 A 0.63 B	0.679	0.690	0.621	0.656	0.598	0.603	0.689	0.695
	0.653	0.677	0.649	0.733	0.633	0.594	0.726	0.651
	0.666	0.684	0.635	0.695	0.616	0.599	0.708	0.673
1.25 A 1.25 B	1.04	1.06	1.00	1.06	0.958	0.968	1.16	1.11
	1.05	1.12	1.09	1.19	1.04	1.00	1.25	1.15
	1.05	1.09	1.05	1.13	0.999	0.984	1.21	1.13
2.50 A 2.50 B	2.23	2.23	2.12	2.23	2.03	1.84	2.31	2.20
	2.13	2.24	2.19	2.40	2.13	2.02	2.42	2.27
	2.18	2.24	2.16	2.32	2.08	1.93	2.37	2.24
5.00 A 5.00 B	4.91	4.90	4.58	5.15	4.39	3.99	4.83	4.83
	4.71	5.18	4.89	5.17	4.71	4.12	5.02	4.91
	4.81	5.04	4.74	5.16	4.55	4.06	4.93	4.87
10.0 A 10.0 B	8.70	9.56	9.22	9.68	8.26	7.81	9.22	9.16
	8.89	9.24	9.18	10.3	8.88	7.80	9.70	9.34
	8.80	9.40	9.20	9.99	8.57	7.81	9.46	9.25
Carbon Filter Effluent	<LOQ	<LOQ	<LOQ	0.0415	0.00868	<LOQ	0.00718	<LOQ
Carbon Filter Effluent Resample	<LOQ							

Table 2
Measured Concentrations of H-28548 in Test Solutions (continued)

Nominal H-28548 Concentration, mg/L (corrected for purity)	Measured H-28548 Concentration (mg/L)						
	Day 48	Day 55	Day 62	Day 69	Day 76	Day 83	Day 90
HWW Control A ^b	ND ^c	ND	ND	ND	ND	ND	ND
HWW Control B	ND	ND	ND	ND	ND	ND	ND
0.63 A	0.720	0.580	0.620	0.631	0.658	0.576	0.627
0.63 B	0.693	0.669	0.636	0.678	0.716	0.598	0.603
	0.707	0.625	0.628	0.655	0.687	0.587	0.615
1.25 A	1.09	1.07	1.05	1.03	1.09	0.934	1.05
1.25 B	1.21	1.05	1.01	1.13	1.16	1.01	1.05
	1.15	1.06	1.03	1.08	1.13	0.972	1.05
2.50 A	2.23	1.97	2.12	2.19	2.15	1.97	2.11
2.50 B	2.29	2.17	2.06	2.20	2.19	2.06	2.10
	2.26	2.07	2.09	2.20	2.17	2.02	2.11
5.00 A	4.81	4.38	4.42	4.84	4.62	4.22	4.41
5.00 B	4.67	4.35	4.44	4.59	4.82	4.56	4.59
	4.74	4.37	4.43	4.72	4.72	4.39	4.50
10.0 A	8.91	8.88	8.20	9.06	8.40	7.90	8.40
10.0 B	8.87	9.38	8.27	8.91	9.58	8.27	8.59
	8.89	9.13	8.24	8.99	8.99	8.09	8.50
Carbon Filter Effluent	<LOQ	0.00588	0.0219	0.0471	<LOQ	0.0731	0.0889
Carbon Filter Effluent Resample						0.257	

Table 2
Measured Concentrations of H-28548 in Test Solutions (continued)

Nominal H-28548 Concentration, mg/L (corrected for purity)	Mean, Measured H-28548 Concentration (mg/L) ^d	Mean Percent Recovery ^e
HW Control A ^b	---	---
HW Control B	---	---
0.63 A 0.63 B	0.651	103
1.25 A 1.25 B	1.08	86
2.50 A 2.50 B	2.16	86
5.00 A 5.00 B	4.66	93
10.0 A 10.0 B	8.89	89
Carbon Filter Effluent	---	---
Carbon Filter Effluent Resample	---	---

- a Day -1 measured values were used to verify the flow-through delivery system prior to study start and were not used in the calculation of mean, measured concentrations.
- b Replicate identification.
- c ND denotes not detected. The limit of detection (LOD) for H-28548 was calculated as 0.009 mg/L after application of a 20× dilution factor. <LOQ denotes below limit of quantitation (LOQ). The limit of quantitation for H-28548 was 0.100 mg/L after application of a 20× dilution factor. For carbon filter effluent samples no dilution was performed prior to analysis resulting in LOD and LOQ values of 0.0005 mg/L and 0.005 mg/L, respectively.
- d Mean, measured H-28548 concentration was calculated as the average of daily mean measured values from day 0 through day 90 within a test concentration.
- e Based on nominal concentration.

Table 3
Water Chemistry of the Dilution Water Control and Highest Test Concentration with Surviving Organisms

Day	Replicate	Total Alkalinity (mg/L as CaCO ₃)	EDTA Hardness (mg/L as CaCO ₃)	Conductivity (µmhos/cm)
Dilution Water Control				
0	A	82	142	238
6	A	81	143	252
13	A	89	143	252
20	A	85	142	250
27	A	87	120	217
34	A	84	146	250
41	A	88	158	240
48	A	85	160	242
55	A	92	138	242
62	A	89	151	251
69	A	89	139	240
76	A	90	100	180
83	A	84	146	250
90	A	83	142	255
Highest Test Concentration with Surviving Organisms - 8.89 ^a mg/L				
0	A	84	148	269
6	A	88	146	265
13	A	85	141	262
20	A	83	144	255
27	A	84	144	247
34	A	82	145	255
41	A	85	156	235
48	A	86	166	242
55	A	84	141	245
62	A	87	152	253
69	A	87	142	245
76	A	88	105	182
83	A	82	142	255
90	A	82	144	260

a Based on mean, measured test concentration.

Table 4
Temperature (°C) of Test Solutions for the 90-Day Rainbow Trout, *Oncorhynchus mykiss*,
Early Life-Stage Toxicity Test

Day	Replicate	Dilution Water Control	Temperature (°C)					Water Bath
			Mean, Measured Concentrations (mg/L)					
			0.651	1.08	2.16	4.66	8.89	
0	A	12.5	12.5	12.5	12.5	12.5	12.7	12.5
0	B	12.5	12.5	12.5	12.5	12.6	12.6	12.5
6	A	12.7	12.6	12.7	12.7	12.8	13.0	12.3
6	B	12.7	12.7	12.8	12.7	12.8	12.9	12.3
13	A	12.5	12.5	12.5	12.6	12.6	12.7	12.7
13	B	12.6	12.5	12.5	12.6	12.6	12.7	12.7
20	A	12.7	12.7	12.7	12.7	12.8	12.8	12.5
20	B	12.7	12.7	12.7	12.7	12.8	12.8	12.5
27	A	12.6	12.6	12.6	12.6	12.7	12.9	12.5
27	B	12.6	12.6	12.6	12.7	12.7	12.8	12.5
34	A	12.4	12.4	12.5	12.5	12.5	12.6	12.5
34	B	12.4	12.4	12.5	12.5	12.5	12.6	12.5
41	A	12.5	12.5	12.5	12.5	12.6	12.7	12.2
41	B	12.5	12.5	12.5	12.5	12.7	12.8	12.2
48	A	12.5	12.6	12.6	12.6	12.7	12.7	12.5
48	B	12.6	12.6	12.5	12.7	12.7	12.7	12.5
55	A	12.5	12.6	12.5	12.5	12.7	12.7	12.5
55	B	12.6	12.6	12.5	12.7	12.7	12.7	12.5
62	A	12.5	12.5	12.4	12.4	12.5	12.5	12.5
62	B	12.6	12.5	12.5	12.5	12.5	12.5	12.5
69	A	12.5	12.5	12.5	12.5	12.6	12.6	12.6
69	B	12.5	12.5	12.5	12.5	12.6	12.6	12.6
76	A	12.4	12.3	12.3	12.3	12.3	12.4	12.2
76	B	12.4	12.3	12.3	12.3	12.3	12.3	12.2
83	A	11.9	11.9	11.8	11.9	11.9	11.9	11.8
83	B	11.9	11.9	11.8	11.9	11.9	11.9	11.8
90	A	12.6	12.6	12.6	12.6	12.6	12.7	12.7
90	B	12.6	12.6	12.6	12.6	12.6	12.7	12.7
Temperature (°C)								
Mean for all concentrations, including controls								12.5
Std. Dev. of for all concentrations, including controls								0.22
Minimum for all concentrations, including controls								11.8
Maximum for all concentrations, including controls								13.0

Table 5
pH of Test Solutions for the 90-Day Rainbow Trout, *Oncorhynchus mykiss*, Early Life-Stage Toxicity Test

Day	Replicate	Dilution Water Control	pH Mean, Measured Concentrations (mg/L)				
			0.651	1.08	2.16	4.66	8.89
0	A	8.2	8.2	8.2	8.2	8.2	8.1
0	B	8.2	8.2	8.2	8.2	8.2	8.2
6	A	7.9	8.0	8.1	8.1	8.1	8.1
6	B	8.0	8.1	8.1	8.1	8.1	8.1
13	A	8.3	8.3	8.3	8.3	8.2	8.1
13	B	8.3	8.3	8.3	8.3	8.2	8.1
20	A	8.1	8.1	8.1	8.1	8.1	8.1
20	B	8.2	8.2	8.2	8.2	8.2	8.2
27	A	8.1	8.1	8.1	8.1	8.1	8.0
27	B	8.1	8.1	8.1	8.1	8.1	8.0
34	A	8.2	8.1	8.1	8.1	8.1	8.0
34	B	8.2	8.2	8.1	8.1	8.1	8.0
41	A	8.0	8.0	8.0	8.0	8.0	8.0
41	B	8.0	8.0	8.0	8.0	8.0	8.0
48	A	7.9	8.0	8.0	8.0	8.0	8.0
48	B	8.0	8.0	8.0	8.1	8.0	7.9
55	A	7.7	7.7	7.7	7.7	7.7	7.7
55	B	7.7	7.7	7.7	7.7	7.7	7.7
62	A	7.5	7.5	7.6	7.5	7.6	7.6
62	B	7.5	7.6	7.6	7.5	7.6	7.6
69	A	7.5	7.5	7.5	7.6	7.6	7.6
69	B	7.5	7.5	7.6	7.6	7.6	7.6
76	A	7.6	7.5	7.5	7.5	7.5	7.4
76	B	7.6	7.5	7.5	7.5	7.5	7.5
83	A	7.4	7.4	7.4	7.5	7.5	7.5
83	B	7.4	7.4	7.4	7.5	7.5	7.5
90	A	7.9	7.9	7.9	7.9	7.9	7.9
90	B	7.9	7.9	7.9	7.9	7.9	7.9
Minimum for all concentrations, including controls							7.4
Maximum for all concentrations, including controls							8.3

Table 6
Dissolved Oxygen Concentration (mg/L) of Test Solutions for the 90-Day Rainbow Trout,
Oncorhynchus mykiss, Early Life-Stage Toxicity Test

Day	Replicate	Dilution Water Control	Dissolved Oxygen Concentration (mg/L) ^a Mean, Measured Concentrations (mg/L)				
			0.651	1.08	2.16	4.66	8.89
0	A	10.3	10.3	10.3	10.2	10.1	10.1
0	B	10.3	10.3	10.2	10.2	10.1	10.1
6	A	10.0	9.7	9.8	9.8	9.8	9.6
6	B	9.8	9.8	9.8	9.7	9.6	9.6
13	A	10.0	10.0	10.1	10.1	10.1	9.6
13	B	10.1	10.0	9.9	10.0	9.9	9.6
20	A	10.0	9.9	9.9	9.8	9.7	9.6
20	B	9.8	9.7	9.8	9.7	9.6	9.6
27	A	10.0	10.0	9.9	9.9	9.8	9.7
27	B	9.9	9.8	9.9	9.8	9.8	9.6
34	A	10.1	10.0	10.0	9.9	9.8	9.6
34	B	10.1	10.0	9.9	9.9	9.7	9.6
41	A	9.8	9.8	9.7	9.6	9.6	9.5
41	B	9.8	9.8	9.7	9.7	9.5	9.3
48	A	9.5	9.4	9.4	9.4	9.4	9.3
48	B	9.5	9.4	9.4	9.4	9.4	9.2
55	A	8.4	8.3	8.0	7.9	7.8	7.6
55	B	8.4	8.1	8.0	7.8	7.8	7.5
62	A	7.9	7.7	7.1	7.1	7.1	6.8
62	B	7.8	7.5	7.1	7.0	7.0	6.7
69	A	7.8	7.6	7.2	7.0	6.9	6.7
69	B	7.7	7.6	7.1	7.0	7.0	6.6
76	A	7.8	6.8	6.2	5.8	6.1	6.4
76	B	7.2	5.8	6.1	6.8	7.0	5.8
83	A	8.0	8.4	7.9	8.5	8.4	7.4
83	B	8.5	8.9	8.4	8.3	7.9	8.3
90	A	8.7	8.9	8.7	8.9	8.2	8.7
90	B	8.5	9.2	8.2	8.9	8.7	8.7
			Dissolved Oxygen Concentration (mg/L)				
			Mean for all concentrations, including controls				
			Std. Dev. for all concentrations, including controls				
			Minimum for all concentrations, including controls				
			Maximum for all concentrations, including controls				

a The theoretical dissolved oxygen concentration at saturation at 12°C = 11.3 mg/L.

Table 7
Summary of Hatching, Survival, Abnormalities, and Swim-Up from Hatching to Thinning for the 90-Day Rainbow Trout, *Oncorhynchus mykiss*, Early Life-Stage Toxicity Test

Mean, Measured H-28548 Concentration (mg/L)Replicate		Hatching Day		Percent Hatch			Hatching to Thinning						First Day of Swim-Up
							Survival			Abnormalities ^b			
		First	Last ^a	Number Hatching	Total	Percent	Number Alive	Number Hatching	Percent	Number Affected	Number Alive	Percent	
Dilution Water Control	A1	21	24	15	20	75.0	10	15	66.7	1	10	10.0	36
	A2	20	24	19	20	95.0	14	19	73.7	0	14	0.0	35
	B1	21	24	17	20	85.0	13	17	76.5	0	13	0.0	37
	B2	21	24	14	20	70.0	13	14	92.9	1	13	7.7	37
	Mean ^c	20.75	24	16.25	20	81.3	12.5	16.25	77.4	0.5	12.5	4.4	36.25
0.651	A1	20	24	14	20	70.0	9	14	64.3	1	9	11.1	39
	A2	21	25	19	20	95.0	13	19	68.4	1	13	7.7	37
	B1	21	24	15	20	75.0	14	15	93.3	1	14	7.1	36
	B2	21	24	20	20	100.0	10	20	50.0	0	10	0.0	38
	Mean ^c	20.75	24.25	17	20	85.0	11.5	17	69.0	1	11.5	9.0	37.5
1.08	A1	21	24	16	20	80.0	12	16	75.0	0	12	0.0	36
	A2	21	24	18	20	90.0	12	18	66.7	0	12	0.0	37
	B1	20	23	16	20	80.0	14	16	87.5	1	14	7.1	37
	B2	20	23	14	20	70.0	9	14	64.3	0	9	0.0	37
	Mean ^c	20.5	23.5	16	20	80.0	11.75	16	73.4	0.25	11.75	1.8	36.75
2.16	A1	21	23	19	20	95.0	16	19	84.2	1	16	6.3	37
	A2	21	23	16	20	80.0	11	16	68.8	0	11	0.0	38
	B1	20	23	17	20	85.0	12	17	70.6	1	12	8.3	36
	B2	21	23	17	20	85.0	11	17	64.7	1	11	9.1	35
	Mean ^c	20.75	23	17.25	20	86.3	12.5	17.25	72.1	0.75	12.5	5.9	36.5

Table 7
Summary of Hatching, Survival, Abnormalities, and Swim-Up from Hatching to Thinning for the 90-Day Rainbow Trout, *Oncorhynchus mykiss*, Early Life-Stage Toxicity Test (Continued)

Mean, Measured H-28548 Concentration (mg/L)Replicate		Hatching Day		Percent Hatch			Hatching to Thinning						First Day of Swim-Up
							Survival			Abnormalities ^b			
		First	Last ^a	Number Hatching	Total	Percent	Number Alive	Number Hatching	Percent	Number Affected	Number Alive	Percent	
4.66	A1	21	23	18	20	90.0	12	18	66.7	0	12	0.0	38
	A2	21	23	16	20	80.0	11	16	68.8	0	11	0.0	37
	B1	20	23	17	20	85.0	10	17	58.8	0	10	0.0	38
	B2	21	23	15	20	75.0	9	15	60.0	0	9	0.0	36
	Mean ^c	20.75	23	16.5	20	82.5	10.5	16.5	63.6	0	10.5	0.0	37.25
8.89	A1	21	23	16	20	80.0	13	16	81.3	0	13	0.0	36
	A2	21	23	16	20	80.0	13	16	81.3	1	13	7.7	35
	B1	20	23	16	20	80.0	12	16	75.0	0	12	0.0	35
	B2	21	23	18	20	90.0	13	18	72.2	1	13	7.7	35
	Mean ^c	20.75	23	16.5	20	82.5	12.75	16.5	77.4	0.5	12.75	3.8	35.25

a Last day of hatching is defined as the last day that remaining live eggs (or live embryos) hatch to yield live alevins.

b Based on abnormalities still present at the time of thinning.

c Calculated as mean of replicate values, percent is based on mean values.

Table 8
Summary of Survival, Abnormalities, and Growth from Thinning to Test End for the 90-Day Rainbow Trout, *Oncorhynchus mykiss*,
Early Life-Stage Toxicity Test

Mean, Measured H-28548 Concentration (mg/L)		Thinning to Test End						Growth of Surviving Fingerlings			
		Survival			Abnormalities ^a			Mean		Mean	
		Number			Number			Standard Length		Wet Weight	
	Replicate	Alive	Total	Percent	Affected	Alive	Percent	± Std. Dev. (cm)		± Std. Dev. (g)	
Dilution Water Control	A	14	15	93.3	0	14	0.0	4.9	± 0.4	1.682	± 0.420
	B	14	15	93.3	1	14	7.1	4.8	± 0.5	1.665	± 0.481
	Mean ^b	14	15	93.3	0.5	14	3.6	4.8	0.4	1.674	0.444
0.651	A	14	15	93.3	0	14	0.0	4.9	± 0.3	1.781	± 0.469
	B	15	15	100.0	0	15	0.0	4.9	± 0.2	1.696	± 0.287
	Mean ^b	14.5	15	96.7	0	14.5	0.0	4.9	0.3	1.737	0.381
1.08	A	15	15	100.0	0	15	0.0	4.9	± 0.2	1.730	± 0.313
	B	14	15	93.3	0	14	0.0	5.1	± 0.2	1.864	± 0.264
	Mean ^b	14.5	15	96.7	0	14.5	0.0	5.0	0.2	1.795	0.293
2.16	A	15	15	100.0	0	15	0.0	4.9	± 0.3	1.697	± 0.286
	B	15	15	100.0	0	15	0.0	4.9	± 0.3	1.698	± 0.338
	Mean ^b	15	15	100.0	0	15	0.0	4.9	0.3	1.698	0.308
4.66	A	15	15	100.0	1	15	6.7	4.8	± 0.3	1.700	± 0.342
	B	15	15	100.0	0	15	0.0	4.7	± 0.4	1.623	± 0.442
	Mean ^b	15	15	100.0	0.5	15	3.3	4.8	0.4	1.661	0.390
8.89	A	15	15	100.0	1	15	6.7	4.9	± 0.3	1.760	± 0.353
	B	15	15	100.0	0	15	0.0	4.9	± 0.2	1.726	± 0.337
	Mean ^b	15	15	100.0	0.5	15	3.3	4.9	0.2	1.743	0.340

a Based on abnormalities still present at test end.

b Calculated as mean of replicate values, percent is based on mean values.

Table 9
Dilution Water Control: Embryo Survival and Hatching Data for the 90-Day Rainbow Trout,
Oncorhynchus mykiss, Early Life-Stage Toxicity Test

Day	Replicate [†]	Dead Eggs Removed	Dead Larvae Removed	Cumulative Dead Eggs	Live Eggs Left	Cumulative Dead Larvae	Live Larvae
0	A1	0	0	0	20	0	0
0	A2	0	0	0	20	0	0
0	B1	0	0	0	20	0	0
0	B2	0	0	0	20	0	0
1	A1	0	0	0	20	0	0
1	A2	0	0	0	20	0	0
1	B1	0	0	0	20	0	0
1	B2	0	0	0	20	0	0
2	A1	0	0	0	20	0	0
2	A2	0	0	0	20	0	0
2	B1	0	0	0	20	0	0
2	B2	0	0	0	20	0	0
3	A1	0	0	0	20	0	0
3	A2	0	0	0	20	0	0
3	B1	0	0	0	20	0	0
3	B2	0	0	0	20	0	0
4	A1	0	0	0	20	0	0
4	A2	0	0	0	20	0	0
4	B1	0	0	0	20	0	0
4	B2	0	0	0	20	0	0
5	A1	0	0	0	20	0	0
5	A2	0	0	0	20	0	0
5	B1	0	0	0	20	0	0
5	B2	0	0	0	20	0	0
6	A1	1	0	1	19	0	0
6	A2	0	0	0	20	0	0
6	B1	0	0	0	20	0	0
6	B2	0	0	0	20	0	0
7	A1	1	0	2	18	0	0
7	A2	0	0	0	20	0	0
7	B1	0	0	0	20	0	0
7	B2	0	0	0	20	0	0

Table 9
Dilution Water Control: Embryo Survival and Hatching Data for the 90-Day Rainbow Trout, *Oncorhynchus mykiss*, Early Life-Stage Toxicity Test (Continued)

Day	Replicate [†]	Dead Eggs Removed	Dead Larvae Removed	Cumulative Dead Eggs	Live Eggs Left	Cumulative Dead Larvae	Live Larvae
8	A1	0	0	2	18	0	0
8	A2	0	0	0	20	0	0
8	B1	0	0	0	20	0	0
8	B2	0	0	0	20	0	0
9	A1	0	0	2	18	0	0
9	A2	0	0	0	20	0	0
9	B1	0	0	0	20	0	0
9	B2	0	0	0	20	0	0
10	A1	0	0	2	18	0	0
10	A2	0	0	0	20	0	0
10	B1	0	0	0	20	0	0
10	B2	1	0	1	19	0	0
11	A1	0	0	2	18	0	0
11	A2	0	0	0	20	0	0
11	B1	0	0	0	20	0	0
11	B2	2	0	3	17	0	0
12	A1	0	0	2	18	0	0
12	A2	0	0	0	20	0	0
12	B1	0	0	0	20	0	0
12	B2	0	0	3	17	0	0
13	A1	0	0	2	18	0	0
13	A2	0	0	0	20	0	0
13	B1	0	0	0	20	0	0
13	B2	0	0	3	17	0	0
14	A1	0	0	2	18	0	0
14	A2	0	0	0	20	0	0
14	B1	0	0	0	20	0	0
14	B2	0	0	3	17	0	0
15	A1	0	0	2	18	0	0
15	A2	0	0	0	20	0	0
15	B1	0	0	0	20	0	0
15	B2	0	0	3	17	0	0

Table 9
Dilution Water Control: Embryo Survival and Hatching Data for the 90-Day Rainbow Trout, *Oncorhynchus mykiss*, Early Life-Stage Toxicity Test (Continued)

Day	Replicate [†]	Dead Eggs Removed	Dead Larvae Removed	Cumulative Dead Eggs	Live Eggs Left	Cumulative Dead Larvae	Live Larvae
16	A1	0	0	2	18	0	0
16	A2	0	0	0	20	0	0
16	B1	0	0	0	20	0	0
16	B2	1	0	4	16	0	0
17	A1	0	0	2	18	0	0
17	A2	0	0	0	20	0	0
17	B1	1	0	1	19	0	0
17	B2	0	0	4	16	0	0
18	A1	0	0	2	18	0	0
18	A2	0	0	0	20	0	0
18	B1	0	0	1	19	0	0
18	B2	0	0	4	16	0	0
19	A1	0	0	2	18	0	0
19	A2	0	0	0	20	0	0
19	B1	0	0	1	19	0	0
19	B2	0	0	4	16	0	0
20	A1	0	0	2	18	0	0
20	A2	0	1	0	18	1	1
20	B1	0	0	1	19	0	0
20	B2	0	0	4	16	0	0
21	A1	0	0	2	16	0	2
21	A2	0	0	0	18	0	2
21	B1	0	0	1	16	0	3
21	B2	0	0	4	9	0	7
22	A1	0	0	2	6	0	12
22	A2	0	1	0	5	1	14
22	B1	1	1	2	3	1	14
22	B2	0	0	4	3	0	13
23	A1	0	0	2	3	0	15
23	A2	1	0	1	2	1	16
23	B1	1	1	3	1	1	15
23	B2	2	0	6	1	0	13

Table 9
Dilution Water Control: Embryo Survival and Hatching Data for the 90-Day Rainbow Trout, *Oncorhynchus mykiss*, Early Life-Stage Toxicity Test (Continued)

Day	Replicate [†]	Dead Eggs Removed	Dead Larvae Removed	Cumulative Dead Eggs	Live Eggs Left	Cumulative Dead Larvae	Live Larvae
24	A1	3	2	5	0	2	13
24	A2	0	1	1	0	2	17
24	B1	0	0	3	0	1	16
24	B2	0	0	6	0	0	14
25	A1	0	0	5	0	2	13
25	A2	0	0	1	0	2	17
25	B1	0	1	3	0	2	15
25	B2	0	0	6	0	0	14
26	A1	0	0	5	0	2	13
26	A2	0	1	1	0	3	16
26	B1	0	0	3	0	2	15
26	B2	0	0	6	0	0	14
27	A1	0	0	5	0	2	13
27	A2	0	1	1	0	4	15
27	B1	0	1	3	0	3	14
27	B2	0	0	6	0	0	14
28	A1	0	0	5	0	2	13
28	A2	0	0	1	0	4	15
28	B1	0	0	3	0	3	14
28	B2	0	0	6	0	0	14
29	A1	0	0	5	0	2	13
29	A2	0	0	1	0	4	15
29	B1	0	0	3	0	3	14
29	B2	0	0	6	0	0	14
30	A1	0	0	5	0	2	13 (1a)
30	A2	0	0	1	0	4	15
30	B1	0	0	3	0	3	14
30	B2	0	0	6	0	0	14
31	A1	0	0	5	0	2	13 (1a,1b) (1ab)
31	A2	0	0	1	0	4	15 (1a)
31	B1	0	0	3	0	3	14 (1a)
31	B2	0	0	6	0	0	14 (1a)

Table 9
Dilution Water Control: Embryo Survival and Hatching Data for the 90-Day Rainbow Trout, *Oncorhynchus mykiss*, Early Life-Stage Toxicity Test (Continued)

Day	Replicate [†]	Dead Eggs Removed	Dead Larvae Removed	Cumulative Dead Eggs	Live Eggs Left	Cumulative Dead Larvae	Live Larvae
32	A1	0	0	5	0	2	13 (1a) (1ab)
32	A2	0	0	1	0	4	15 (1a)
32	B1	0	0	3	0	3	14 (1a)
32	B2	0	0	6	0	0	14 (1a)
33	A1	0	0	5	0	2	13 (1a) (1ab)
33	A2	0	0	1	0	4	15 (1a)
33	B1	0	0	3	0	3	14 (1ad)
33	B2	0	0	6	0	0	14 (1a)
34	A1	0	1	5	0	3	12 (1bc) (1c, 1ad)
34	A2	0	0	1	0	4	15 (1ad)
34	B1	0	0	3	0	3	14 (1aed)
34	B2	0	0	6	0	0	14 (1bc)
35	A1	0	0	5	0	3	12 (1bc, 1ad) (1cd)
35	A2	0	0	1	0	4	15 (1ad)
35	B1	0	0	3	0	3	14 (1ade)
35	B2	0	0	6	0	0	14 (1bc)
36	A1	0	0	5	0	3	12 (1bc, 1ad) (1cd)
36	A2	0	0	1	0	4	15 (1ad)
36	B1	0	0	3	0	3	14 (1ade)
36	B2	0	0	6	0	0	14 (1bc)
37	A1	0	0	5	0	3	12 (1bc, 1ae) (1ce)
37	A2	0	0	1	0	4	15 (1ad)
37	B1	0	0	3	0	3	14 (1ade)
37	B2	0	0	6	0	0	14 (1bc)
38	A1	0	1	5	0	4	11 (1bce) (1ae)
38	A2	0	0	1	0	4	15 (1ade)
38	B1	0	0	3	0	3	14 (1ade)
38	B2	0	0	6	0	0	14 (1bc) (1a)
39	A1	0	0	5	0	4	11 (1bce) (1ae)
39	A2	0	1	1	0	5	14
39	B1	0	1	3	0	4	13
39	B2	0	0	6	0	0	14 (1bc) (1ae)

Table 9
Dilution Water Control: Embryo Survival and Hatching Data for the 90-Day Rainbow Trout, *Oncorhynchus mykiss*, Early Life-Stage Toxicity Test (Continued)

Day	Replicate [†]	Dead Eggs Removed	Dead Larvae Removed	Cumulative Dead Eggs	Live Eggs Left	Cumulative Dead Larvae	Live Larvae
40	A1	0	0	5	0	4	11 (1bc) (1ae)
40	A2	0	0	1	0	5	14
40	B1	0	0	3	0	4	13
40	B2	0	0	6	0	0	14 (1bce) (1ae)
41	A1	0	0	5	0	4	11 (1bceg) (1aeg)
41	A2	0	0	1	0	5	14
41	B1	0	0	3	0	4	13
41	B2	0	0	6	0	0	14 (1aeg) (1bceg)
42	A1	0	1	5	0	5	10 (1aeg)
42	A2	0	0	1	0	5	14
42	B1	0	0	3	0	4	13
42	B2	0	0	6	0	0	14 (1aeg) (1bceg)
43	A1	0	0	5	0	5	10 (1aeg)
43	A2	0	0	1	0	5	14
43	B1	0	0	3	0	4	13
43	B2	0	0	6	0	0	14 (1aefgh) (1bceg)
44	A1	0	0	5	0	5	10 (1acg)
44	A2	0	0	1	0	5	14
44	B1	0	0	3	0	4	13
44	B2	0	0	6	0	0	14 (1aefgh) (1bceg)
45	A1	0	0	5	0	5	10 (1acg)
45	A2	0	0	1	0	5	14
45	B1	0	0	3	0	4	13
45	B2	0	1	6	0	1	13 (1bceg)

Table 9
Dilution Water Control: Embryo Survival and Hatching Data for the 90-Day Rainbow Trout, *Oncorhynchus mykiss*, Early Life-Stage Toxicity Test (Continued)

Day	Replicate [†]	Dead Eggs Removed	Dead Larvae Removed	Cumulative Dead Eggs	Live Eggs Left	Cumulative Dead Larvae	Live Larvae
46	A1	0	0	5	0	5	10 (1acg)
46	A2	0	0	1	0	5	14
46	B1	0	0	3	0	4	13
46	B2	0	0	6	0	1	13 (1bceg)

[†] Four replicates per concentration (i.e., A1 - B2).

- a small in size (when compared to appropriate control(s))
- b scoliosis (includes: curved spine, corkscrew tail, and bent spine/tail)
- c mouth - potential jaw deformity
- d pale in color
- e lethargic
- f labored respiration (gasping)
- g yolk sac present (not absorbed)
- h lying on bottom of test chamber

Table 10
0.651 mg/L: Embryo Survival and Hatching Data for the 90-Day Rainbow Trout,
Oncorhynchus mykiss, Early Life-Stage Toxicity Test

Day	Replicate [†]	Dead Eggs Removed	Dead Larvae Removed	Cumulative Dead Eggs	Live Eggs Left	Cumulative Dead Larvae	Live Larvae
0	A1	0	0	0	20	0	0
0	A2	0	0	0	20	0	0
0	B1	0	0	0	20	0	0
0	B2	0	0	0	20	0	0
1	A1	0	0	0	20	0	0
1	A2	0	0	0	20	0	0
1	B1	0	0	0	20	0	0
1	B2	0	0	0	20	0	0
2	A1	0	0	0	20	0	0
2	A2	0	0	0	20	0	0
2	B1	0	0	0	20	0	0
2	B2	0	0	0	20	0	0
3	A1	0	0	0	20	0	0
3	A2	0	0	0	20	0	0
3	B1	0	0	0	20	0	0
3	B2	0	0	0	20	0	0
4	A1	0	0	0	20	0	0
4	A2	0	0	0	20	0	0
4	B1	0	0	0	20	0	0
4	B2	0	0	0	20	0	0
5	A1	0	0	0	20	0	0
5	A2	0	0	0	20	0	0
5	B1	0	0	0	20	0	0
5	B2	0	0	0	20	0	0
6	A1	0	0	0	20	0	0
6	A2	0	0	0	20	0	0
6	B1	0	0	0	20	0	0
6	B2	0	0	0	20	0	0
7	A1	0	0	0	20	0	0
7	A2	0	0	0	20	0	0
7	B1	0	0	0	20	0	0
7	B2	0	0	0	20	0	0

Table 10
0.651 mg/L: Embryo Survival and Hatching Data for the 90-Day Rainbow Trout,
Oncorhynchus mykiss, Early Life-Stage Toxicity Test (Continued)

Day	Replicate [†]	Dead Eggs Removed	Dead Larvae Removed	Cumulative Dead Eggs	Live Eggs Left	Cumulative Dead Larvae	Live Larvae
8	A1	0	0	0	20	0	0
8	A2	0	0	0	20	0	0
8	B1	0	0	0	20	0	0
8	B2	0	0	0	20	0	0
9	A1	0	0	0	20	0	0
9	A2	0	0	0	20	0	0
9	B1	0	0	0	20	0	0
9	B2	0	0	0	20	0	0
10	A1	0	0	0	20	0	0
10	A2	0	0	0	20	0	0
10	B1	0	0	0	20	0	0
10	B2	0	0	0	20	0	0
11	A1	0	0	0	20	0	0
11	A2	0	0	0	20	0	0
11	B1	0	0	0	20	0	0
11	B2	0	0	0	20	0	0
12	A1	0	0	0	20	0	0
12	A2	0	0	0	20	0	0
12	B1	0	0	0	20	0	0
12	B2	0	0	0	20	0	0
13	A1	0	0	0	20	0	0
13	A2	0	0	0	20	0	0
13	B1	0	0	0	20	0	0
13	B2	0	0	0	20	0	0
14	A1	0	0	0	20	0	0
14	A2	0	0	0	20	0	0
14	B1	0	0	0	20	0	0
14	B2	0	0	0	20	0	0
15	A1	0	0	0	20	0	0
15	A2	0	0	0	20	0	0
15	B1	0	0	0	20	0	0
15	B2	0	0	0	20	0	0

Table 10
0.651 mg/L: Embryo Survival and Hatching Data for the 90-Day Rainbow Trout,
Oncorhynchus mykiss, Early Life-Stage Toxicity Test (Continued)

Day	Replicate [†]	Dead Eggs Removed	Dead Larvae Removed	Cumulative Dead Eggs	Live Eggs Left	Cumulative Dead Larvae	Live Larvae
16	A1	0	0	0	20	0	0
16	A2	0	0	0	20	0	0
16	B1	1	0	1	19	0	0
16	B2	0	0	0	20	0	0
17	A1	0	0	0	20	0	0
17	A2	1	0	1	19	0	0
17	B1	0	0	1	19	0	0
17	B2	0	0	0	20	0	0
18	A1	0	0	0	20	0	0
18	A2	0	0	1	19	0	0
18	B1	0	0	1	19	0	0
18	B2	0	0	0	20	0	0
19	A1	1	0	1	19	0	0
19	A2	0	0	1	19	0	0
19	B1	0	0	1	19	0	0
19	B2	0	0	0	20	0	0
20	A1	0	0	1	18	0	1
20	A2	0	0	1	19	0	0
20	B1	1	0	2	18	0	0
20	B2	0	0	0	20	0	0
21	A1	0	0	1	16	0	3
21	A2	0	0	1	14	0	5
21	B1	0	0	2	13	0	5
21	B2	0	0	0	12	0	8
22	A1	1	0	2	7	0	11
22	A2	0	0	1	6	0	13
22	B1	0	0	2	4	0	14
22	B2	0	0	0	2	0	18
23	A1	3	0	5	4	0	11
23	A2	0	1	1	3	1	15
23	B1	0	0	2	3	0	15
23	B2	0	4	0	2	4	14

Table 10
0.651 mg/L: Embryo Survival and Hatching Data for the 90-Day Rainbow Trout,
Oncorhynchus mykiss, Early Life-Stage Toxicity Test (Continued)

Day	Replicate [†]	Dead Eggs Removed	Dead Larvae Removed	Cumulative Dead Eggs	Live Eggs Left	Cumulative Dead Larvae	Live Larvae
24	A1	1	1	6	0	1	13
24	A2	0	1	1	1	2	16
24	B1	3	0	5	0	0	15
24	B2	0	1	0	0	5	15
25	A1	0	0	6	0	1	13
25	A2	0	0	1	0	2	17
25	B1	0	0	5	0	0	15
25	B2	0	0	0	0	5	15
26	A1	0	2	6	0	3	11
26	A2	0	1	1	0	3	16
26	B1	0	0	5	0	0	15
26	B2	0	1	0	0	6	14
27	A1	0	0	6	0	3	11
27	A2	0	1	1	0	4	15
27	B1	0	0	5	0	0	15
27	B2	0	1	0	0	7	13
28	A1	0	1	6	0	4	10
28	A2	0	0	1	0	4	15
28	B1	0	0	5	0	0	15
28	B2	0	0	0	0	7	13
29	A1	0	0	6	0	4	10
29	A2	0	0	1	0	4	15
29	B1	0	0	5	0	0	15
29	B2	0	0	0	0	7	13
30	A1	0	0	6	0	4	10 (1a,1b)
30	A2	0	0	1	0	4	15
30	B1	0	0	5	0	0	15
30	B2	0	0	0	0	7	13
31	A1	0	0	6	0	4	10 (1a,1b)
31	A2	0	0	1	0	4	15 (1a,1b)
31	B1	0	0	5	0	0	15 (1b)
31	B2	0	0	0	0	7	13

Table 10
0.651 mg/L: Embryo Survival and Hatching Data for the 90-Day Rainbow Trout,
Oncorhynchus mykiss, Early Life-Stage Toxicity Test (Continued)

Day	Replicate [†]	Dead Eggs Removed	Dead Larvae Removed	Cumulative Dead Eggs	Live Eggs Left	Cumulative Dead Larvae	Live Larvae
32	A1	0	0	6	0	4	10 (1a)
32	A2	0	0	1	0	4	15 (1b)
32	B1	0	0	5	0	0	15 (1a)
32	B2	0	0	0	0	7	13 (1b)
33	A1	0	0	6	0	4	10 (1a)
33	A2	0	0	1	0	4	15 (1b)
33	B1	0	0	5	0	0	15 (1a)
33	B2	0	0	0	0	7	13 (1b)
34	A1	0	0	6	0	4	10 (1ac)
34	A2	0	1	1	0	5	14 (1b)
34	B1	0	0	5	0	0	15 (1c)
34	B2	0	0	0	0	7	13 (1c)
35	A1	0	0	6	0	4	10 (3c)
35	A2	0	0	1	0	5	14 (1acd)
35	B1	0	0	5	0	0	15 (1b)
35	B2	0	0	0	0	7	13 (1cd)
36	A1	0	0	6	0	4	10 (1b)
36	A2	0	0	1	0	5	14 (1b)
36	B1	0	0	5	0	0	15 (1cd)
36	B2	0	0	0	0	7	13 (1cd)
37	A1	0	0	6	0	4	10 (3cd)
37	A2	0	0	1	0	5	14 (1acd)
37	B1	0	0	5	0	0	15 (1b)
37	B2	0	0	0	0	7	13 (1cd)
38	A1	0	1	6	0	5	9 (1b)
38	A2	0	0	1	0	5	14 (1b)
38	B1	0	0	5	0	0	15 (1cd)
38	B2	0	0	0	0	7	13 (1cd)

Table 10
0.651 mg/L: Embryo Survival and Hatching Data for the 90-Day Rainbow Trout,
Oncorhynchus mykiss, Early Life-Stage Toxicity Test (Continued)

Day	Replicate [†]	Dead Eggs Removed	Dead Larvae Removed	Cumulative Dead Eggs	Live Eggs Left	Cumulative Dead Larvae	Live Larvae
39	A1	0	0	6	0	5	9 (1bd)
39	A2	0	0	1	0	5	14 (1b)
39	B1	0	0	5	0	0	15 (1cd)
39	B2	0	0	0	0	7	13 (3cd)
40	A1	0	0	6	0	5	9 (1d)
40	A2	0	1	1	0	6	9 (1be)
40	B1	0	0	5	0	0	13 (1b)
40	B2	0	0	0	0	7	15 (1cd)
41	A1	0	0	6	0	5	13 (3cd)
41	A2	0	0	1	0	6	13 (1d)
41	B1	0	0	5	0	0	9 (1bde)
41	B2	0	0	0	0	7	13 (1b)
42	A1	0	0	6	0	5	15 (1bf)
42	A2	0	0	1	0	6	15 (1cdf)
42	B1	0	0	5	0	0	13 (3cdf)
42	B2	0	1	0	0	8	12 (1bde)
43	A1	0	0	6	0	5	13 (1ab)
43	A2	0	0	1	0	6	15 (1bf)
43	B1	0	0	5	0	0	15 (1cdf)
43	B2	0	0	0	0	8	12 (2cdf)
44	A1	0	0	6	0	5	9 (1bde)
44	A2	0	0	1	0	6	13 (1abe)
44	B1	0	0	5	0	0	15 (1bdf)
44	B2	0	0	0	0	8	12 (1cdf)

Table 10
0.651 mg/L: Embryo Survival and Hatching Data for the 90-Day Rainbow Trout,
Oncorhynchus mykiss, Early Life-Stage Toxicity Test (Continued)

Day	Replicate [†]	Dead Eggs Removed	Dead Larvae Removed	Cumulative Dead Eggs	Live Eggs Left	Cumulative Dead Larvae	Live Larvae
45	A1	0	0	6	0	5	9 (1bde)
45	A2	0	0	1	0	6	13 (1abe)
45	B1	0	0	5	0	0	15 (1bdf)
45	B2	0	1	0	0	9	11 (1cdf)
46	A1	0	0	6	0	5	9 (1de)
46	A2	0	0	1	0	6	13 (1abe)
46	B1	0	1	5	0	1	14 (1bdf)
46	B2	0	1	0	0	10	10

[†] Four replicates per concentration (i.e., A1 - B2).

- a small in size (when compared to appropriate control(s))
- b scoliosis (includes: curved spine, corkscrew tail, and bent spine/tail)
- c mouth - potential jaw deformity
- d lethargic
- e labored respiration (gasping)
- f yolk sac present (not absorbed)

Table 11
1.08 mg/L: Embryo Survival and Hatching Data for the 90-Day Rainbow Trout,
Oncorhynchus mykiss, Early Life-Stage Toxicity Test

Day	Replicate [†]	Dead Eggs Removed	Dead Larvae Removed	Cumulative Dead Eggs	Live Eggs Left	Cumulative Dead Larvae	Live Larvae
0	A1	0	0	0	20	0	0
0	A2	0	0	0	20	0	0
0	B1	0	0	0	20	0	0
0	B2	0	0	0	20	0	0
1	A1	0	0	0	20	0	0
1	A2	0	0	0	20	0	0
1	B1	0	0	0	20	0	0
1	B2	0	0	0	20	0	0
2	A1	0	0	0	20	0	0
2	A2	0	0	0	20	0	0
2	B1	0	0	0	20	0	0
2	B2	0	0	0	20	0	0
3	A1	0	0	0	20	0	0
3	A2	0	0	0	20	0	0
3	B1	0	0	0	20	0	0
3	B2	0	0	0	20	0	0
4	A1	0	0	0	20	0	0
4	A2	0	0	0	20	0	0
4	B1	0	0	0	20	0	0
4	B2	0	0	0	20	0	0
5	A1	1	0	1	19	0	0
5	A2	0	0	0	20	0	0
5	B1	0	0	0	20	0	0
5	B2	0	0	0	20	0	0
6	A1	0	0	1	19	0	0
6	A2	0	0	0	20	0	0
6	B1	0	0	0	20	0	0
6	B2	0	0	0	20	0	0
7	A1	0	0	1	19	0	0
7	A2	0	0	0	20	0	0
7	B1	0	0	0	20	0	0
7	B2	0	0	0	20	0	0

Table 11
1.08 mg/L: Embryo Survival and Hatching Data for the 90-Day Rainbow Trout,
Oncorhynchus mykiss, Early Life-Stage Toxicity Test (Continued)

Day	Replicate [†]	Dead Eggs Removed	Dead Larvae Removed	Cumulative Dead Eggs	Live Eggs Left	Cumulative Dead Larvae	Live Larvae
8	A1	0	0	1	19	0	0
8	A2	0	0	0	20	0	0
8	B1	0	0	0	20	0	0
8	B2	0	0	0	20	0	0
9	A1	0	0	1	19	0	0
9	A2	0	0	0	20	0	0
9	B1	0	0	0	20	0	0
9	B2	0	0	0	20	0	0
10	A1	0	0	1	19	0	0
10	A2	0	0	0	20	0	0
10	B1	0	0	0	20	0	0
10	B2	0	0	0	20	0	0
11	A1	0	0	1	19	0	0
11	A2	0	0	0	20	0	0
11	B1	0	0	0	20	0	0
11	B2	1	0	1	19	0	0
12	A1	0	0	1	19	0	0
12	A2	0	0	0	20	0	0
12	B1	0	0	0	20	0	0
12	B2	0	0	1	19	0	0
13	A1	0	0	1	19	0	0
13	A2	0	0	0	20	0	0
13	B1	0	0	0	20	0	0
13	B2	0	0	1	19	0	0
14	A1	0	0	1	19	0	0
14	A2	0	0	0	20	0	0
14	B1	0	0	0	20	0	0
14	B2	0	0	1	19	0	0
15	A1	0	0	1	19	0	0
15	A2	0	0	0	20	0	0
15	B1	0	0	0	20	0	0
15	B2	1	0	2	18	0	0

Table 11
1.08 mg/L: Embryo Survival and Hatching Data for the 90-Day Rainbow Trout,
Oncorhynchus mykiss, Early Life-Stage Toxicity Test (Continued)

Day	Replicate [†]	Dead Eggs Removed	Dead Larvae Removed	Cumulative Dead Eggs	Live Eggs Left	Cumulative Dead Larvae	Live Larvae
16	A1	0	0	1	19	0	0
16	A2	0	0	0	20	0	0
16	B1	0	0	0	20	0	0
16	B2	0	0	2	18	0	0
17	A1	0	0	1	19	0	0
17	A2	0	0	0	20	0	0
17	B1	0	0	0	20	0	0
17	B2	0	0	2	18	0	0
18	A1	0	0	1	19	0	0
18	A2	0	0	0	20	0	0
18	B1	0	0	0	20	0	0
18	B2	1	0	3	17	0	0
19	A1	2	0	3	17	0	0
19	A2	0	0	0	20	0	0
19	B1	0	0	0	20	0	0
19	B2	1	0	4	16	0	0
20	A1	0	0	3	17	0	0
20	A2	0	0	0	20	0	0
20	B1	0	1	0	19	1	0
20	B2	0	0	4	15	0	1
21	A1	0	0	3	14	0	3
21	A2	0	0	0	15	0	5
21	B1	0	0	0	16	1	3
21	B2	1	0	5	10	0	5
22	A1	0	0	3	4	0	13
22	A2	0	0	0	4	0	16
22	B1	0	0	1	7	0	12
22	B2	0	0	5	1	0	14
23	A1	0	0	3	3	0	14
23	A2	2	1	2	1	1	16
23	B1	3	0	4	0	0	16
23	B2	1	0	6	0	0	14

Table 11
1.08 mg/L: Embryo Survival and Hatching Data for the 90-Day Rainbow Trout,
Oncorhynchus mykiss, Early Life-Stage Toxicity Test (Continued)

Day	Replicate [†]	Dead Eggs Removed	Dead Larvae Removed	Cumulative Dead Eggs	Live Eggs Left	Cumulative Dead Larvae	Live Larvae
24	A1	1	1	4	0	1	15
24	A2	0	1	2	0	2	16
24	B1	0	0	4	0	0	16
24	B2	0	3	6	0	3	11
25	A1	0	1	4	0	2	14
25	A2	0	2	2	0	4	14
25	B1	0	0	4	0	0	16
25	B2	0	0	6	0	3	11
26	A1	0	0	4	0	2	14
26	A2	0	0	2	0	4	14
26	B1	0	1	4	0	1	15
26	B2	0	0	6	0	3	11
27	A1	0	0	4	0	2	14
27	A2	0	0	2	0	4	14
27	B1	0	0	4	0	1	15
27	B2	0	1	6	0	4	10
28	A1	0	0	4	0	2	14
28	A2	0	0	2	0	4	14
28	B1	0	0	4	0	1	15
28	B2	0	0	6	0	4	10
29	A1	0	0	4	0	2	14
29	A2	0	0	2	0	4	14
29	B1	0	0	4	0	1	15
29	B2	0	0	6	0	4	10
30	A1	0	0	4	0	2	14
30	A2	0	0	2	0	4	14
30	B1	0	1	4	0	2	14
30	B2	0	0	6	0	4	10
31	A1	0	0	4	0	2	14
31	A2	0	0	2	0	4	14
31	B1	0	0	4	0	2	14
31	B2	0	0	6	0	4	10

(1ab)
(1ac)

(1ab)
(1ac)

(1b)

Table 11
1.08 mg/L: Embryo Survival and Hatching Data for the 90-Day Rainbow Trout,
Oncorhynchus mykiss, Early Life-Stage Toxicity Test (Continued)

Day	Replicate [†]	Dead Eggs Removed	Dead Larvae Removed	Cumulative Dead Eggs	Live Eggs Left	Cumulative Dead Larvae	Live Larvae
32	A1	0	0	4	0	2	14 (1ac)
32	A2	0	0	2	0	4	14 (1ab)
32	B1	0	0	4	0	2	14 (1ac)
32	B2	0	0	6	0	4	10 (1b)
33	A1	0	0	4	0	2	14 (1ac)
33	A2	0	0	2	0	4	14 (1ab)
33	B1	0	0	4	0	2	14 (1ac)
33	B2	0	0	6	0	4	10 (1b)
34	A1	0	0	4	0	2	14 (1ace)
34	A2	0	0	2	0	4	14 (1c)
34	B1	0	0	4	0	2	14 (1abce)
34	B2	0	0	6	0	4	10 (1ace)
35	A1	0	0	4	0	2	14 (1bc)
35	A2	0	0	2	0	4	14 (1ace)
35	B1	0	0	4	0	2	14 (1c)
35	B2	0	0	6	0	4	10 (1abce)
36	A1	0	0	4	0	2	14 (1ace)
36	A2	0	0	2	0	4	14 (1c)
36	B1	0	0	4	0	2	14 (1abce)
36	B2	0	0	6	0	4	10 (1ace)
37	A1	0	0	4	0	2	14 (1b)
37	A2	0	0	2	0	4	14 (1bc)
37	B1	0	0	4	0	2	14 (1ace)
37	B2	0	0	6	0	4	10 (1b)
38	A1	0	1	4	0	3	13 (1bc)
38	A2	0	1	2	0	5	13 (1e)
38	B1	0	0	4	0	2	14 (1ace)
38	B2	0	0	6	0	4	10 (1b)
39	A1	0	0	4	0	3	13 (1bc)
39	A2	0	1	2	0	6	12 (1e)
39	B1	0	0	4	0	2	14 (1ace)
39	B2	0	0	6	0	4	10 (1b)

Table 11
1.08 mg/L: Embryo Survival and Hatching Data for the 90-Day Rainbow Trout,
Oncorhynchus mykiss, Early Life-Stage Toxicity Test (Continued)

Day	Replicate [†]	Dead Eggs Removed	Dead Larvae Removed	Cumulative Dead Eggs	Live Eggs Left	Cumulative Dead Larvae	Live Larvae
40	A1	0	0	4	0	3	13 (1ce)
40	A2	0	0	2	0	6	12
40	B1	0	0	4	0	2	14 (1bg)
40	B2	0	0	6	0	4	10 (1bc) (1e)
41	A1	0	0	4	0	3	13 (1ceh)
41	A2	0	0	2	0	6	12
41	B1	0	0	4	0	2	14 (1beg)
41	B2	0	0	6	0	4	10 (1bceh) (1eh)
42	A1	0	1	4	0	4	12
42	A2	0	0	2	0	6	12
42	B1	0	0	4	0	2	14 (1befi)
42	B2	0	0	6	0	4	10 (1bceh) (1eh)
43	A1	0	0	4	0	4	12
43	A2	0	0	2	0	6	12
43	B1	0	0	4	0	2	14 (1efi)
43	B2	0	0	6	0	4	10 (1bcdeh)
44	A1	0	0	4	0	4	12
44	A2	0	0	2	0	6	12
44	B1	0	0	4	0	2	14 (1efi)
44	B2	0	0	6	0	4	10 (1bcdeh)
45	A1	0	0	4	0	4	12
45	A2	0	0	2	0	6	12
45	B1	0	0	4	0	2	14 (1efi)
45	B2	0	1	6	0	5	9

Table 11
1.08 mg/L: Embryo Survival and Hatching Data for the 90-Day Rainbow Trout,
Oncorhynchus mykiss, Early Life-Stage Toxicity Test (Continued)

Day	Replicate [†]	Dead Eggs Removed	Dead Larvae Removed	Cumulative Dead Eggs	Live Eggs Left	Cumulative Dead Larvae	Live Larvae
46	A1	0	0	4	0	4	12
46	A2	0	0	2	0	6	12
46	B1	0	0	4	0	2	14 (1efi)
46	B2	0	0	6	0	5	9

[†] Four replicates per concentration (i.e., A1 - B2).

- a small in size (when compared to appropriate control(s))
- b scoliosis (includes: curved spine, corkscrew tail, and bent spine/tail)
- c mouth - potential jaw deformity
- d pale in color
- e lethargic
- f labored respiration (gasping)
- g loss of equilibrium
- h yolk sac present (not absorbed)
- i lying on bottom of test chamber

Table 12
2.16 mg/L: Embryo Survival and Hatching Data for the 90-Day Rainbow Trout,
Oncorhynchus mykiss, Early Life-Stage Toxicity Test

Day	Replicate [†]	Dead Eggs Removed	Dead Larvae Removed	Cumulative Dead Eggs	Live Eggs Left	Cumulative Dead Larvae	Live Larvae
0	A1	0	0	0	20	0	0
0	A2	0	0	0	20	0	0
0	B1	0	0	0	20	0	0
0	B2	0	0	0	20	0	0
1	A1	0	0	0	20	0	0
1	A2	0	0	0	20	0	0
1	B1	0	0	0	20	0	0
1	B2	0	0	0	20	0	0
2	A1	0	0	0	20	0	0
2	A2	0	0	0	20	0	0
2	B1	0	0	0	20	0	0
2	B2	0	0	0	20	0	0
3	A1	0	0	0	20	0	0
3	A2	0	0	0	20	0	0
3	B1	0	0	0	20	0	0
3	B2	0	0	0	20	0	0
4	A1	0	0	0	20	0	0
4	A2	0	0	0	20	0	0
4	B1	0	0	0	20	0	0
4	B2	0	0	0	20	0	0
5	A1	0	0	0	20	0	0
5	A2	0	0	0	20	0	0
5	B1	0	0	0	20	0	0
5	B2	0	0	0	20	0	0
6	A1	0	0	0	20	0	0
6	A2	0	0	0	20	0	0
6	B1	0	0	0	20	0	0
6	B2	0	0	0	20	0	0
7	A1	0	0	0	20	0	0
7	A2	0	0	0	20	0	0
7	B1	0	0	0	20	0	0
7	B2	1	0	1	19	0	0

Table 12
2.16 mg/L: Embryo Survival and Hatching Data for the 90-Day Rainbow Trout,
Oncorhynchus mykiss, Early Life-Stage Toxicity Test (Continued)

Day	Replicate [†]	Dead Eggs Removed	Dead Larvae Removed	Cumulative Dead Eggs	Live Eggs Left	Cumulative Dead Larvae	Live Larvae
8	A1	0	0	0	20	0	0
8	A2	0	0	0	20	0	0
8	B1	0	0	0	20	0	0
8	B2	0	0	1	19	0	0
9	A1	0	0	0	20	0	0
9	A2	0	0	0	20	0	0
9	B1	0	0	0	20	0	0
9	B2	0	0	1	19	0	0
10	A1	0	0	0	20	0	0
10	A2	0	0	0	20	0	0
10	B1	0	0	0	20	0	0
10	B2	0	0	1	19	0	0
11	A1	0	0	0	20	0	0
11	A2	0	0	0	20	0	0
11	B1	1	0	1	19	0	0
11	B2	0	0	1	19	0	0
12	A1	0	0	0	20	0	0
12	A2	0	0	0	20	0	0
12	B1	0	0	1	19	0	0
12	B2	1	0	2	18	0	0
13	A1	0	0	0	20	0	0
13	A2	0	0	0	20	0	0
13	B1	0	0	1	19	0	0
13	B2	0	0	2	18	0	0
14	A1	0	0	0	20	0	0
14	A2	0	0	0	20	0	0
14	B1	0	0	1	19	0	0
14	B2	0	0	2	18	0	0
15	A1	0	0	0	20	0	0
15	A2	0	0	0	20	0	0
15	B1	0	0	1	19	0	0
15	B2	0	0	2	18	0	0

Table 12
2.16 mg/L: Embryo Survival and Hatching Data for the 90-Day Rainbow Trout,
Oncorhynchus mykiss, Early Life-Stage Toxicity Test (Continued)

Day	Replicate [†]	Dead Eggs Removed	Dead Larvae Removed	Cumulative Dead Eggs	Live Eggs Left	Cumulative Dead Larvae	Live Larvae
16	A1	0	0	0	20	0	0
16	A2	0	0	0	20	0	0
16	B1	0	0	1	19	0	0
16	B2	0	0	2	18	0	0
17	A1	0	0	0	20	0	0
17	A2	0	0	0	20	0	0
17	B1	0	0	1	19	0	0
17	B2	0	0	2	18	0	0
18	A1	0	0	0	20	0	0
18	A2	0	0	0	20	0	0
18	B1	0	0	1	19	0	0
18	B2	0	0	2	18	0	0
19	A1	0	0	0	20	0	0
19	A2	0	0	0	20	0	0
19	B1	0	0	1	19	0	0
19	B2	0	0	2	18	0	0
20	A1	0	0	0	20	0	0
20	A2	0	0	0	20	0	0
20	B1	0	0	1	18	0	1
20	B2	0	0	2	18	0	0
21	A1	0	0	0	14	0	6
21	A2	0	0	0	16	0	4
21	B1	0	0	1	12	0	7
21	B2	0	0	2	14	0	4
22	A1	1	0	1	4	0	15
22	A2	0	0	0	7	0	13
22	B1	0	0	1	2	0	17
22	B2	0	0	2	1	0	17
23	A1	0	2	1	0	2	17
23	A2	4	0	4	0	0	16
23	B1	2	2	3	0	2	15
23	B2	1	4	3	0	4	13

Table 12
2.16 mg/L: Embryo Survival and Hatching Data for the 90-Day Rainbow Trout,
Oncorhynchus mykiss, Early Life-Stage Toxicity Test (Continued)

Day	Replicate [†]	Dead Eggs Removed	Dead Larvae Removed	Cumulative Dead Eggs	Live Eggs Left	Cumulative Dead Larvae	Live Larvae
24	A1	0	0	1	0	2	17
24	A2	0	3	4	0	3	13
24	B1	0	2	3	0	4	13
24	B2	0	1	3	0	5	12
25	A1	0	0	1	0	2	17
25	A2	0	1	4	0	4	12
25	B1	0	0	3	0	4	13
25	B2	0	0	3	0	5	12
26	A1	0	0	1	0	2	17
26	A2	0	0	4	0	4	12
26	B1	0	0	3	0	4	13
26	B2	0	0	3	0	5	12
27	A1	0	0	1	0	2	17
27	A2	0	0	4	0	4	12
27	B1	0	0	3	0	4	13
27	B2	0	0	3	0	5	12
28	A1	0	0	1	0	2	17
28	A2	0	0	4	0	4	12
28	B1	0	0	3	0	4	13
28	B2	0	0	3	0	5	12
29	A1	0	0	1	0	2	17
29	A2	0	0	4	0	4	12
29	B1	0	0	3	0	4	13
29	B2	0	1	3	0	6	11
30	A1	0	0	1	0	2	17
30	A2	0	0	4	0	4	12 (1d)
30	B1	0	0	3	0	4	13
30	B2	0	0	3	0	6	11
31	A1	0	0	1	0	2	17
31	A2	0	0	4	0	4	12 (1d)
31	B1	0	0	3	0	4	13
31	B2	0	0	3	0	6	11 (1b)

Table 12
2.16 mg/L: Embryo Survival and Hatching Data for the 90-Day Rainbow Trout,
Oncorhynchus mykiss, Early Life-Stage Toxicity Test (Continued)

Day	Replicate [†]	Dead Eggs Removed	Dead Larvae Removed	Cumulative Dead Eggs	Live Eggs Left	Cumulative Dead Larvae	Live Larvae
32	A1	0	0	1	0	2	17
32	A2	0	0	4	0	4	12 (1d)
32	B1	0	0	3	0	4	13 (1a)
32	B2	0	0	3	0	6	11 (1b)
33	A1	0	0	1	0	2	17
33	A2	0	0	4	0	4	12 (1d)
33	B1	0	0	3	0	4	13
33	B2	0	0	3	0	6	11
34	A1	0	0	1	0	2	17
34	A2	0	0	4	0	4	12 (1d) (1c)
34	B1	0	0	3	0	4	13 (1d) (1c)
34	B2	0	0	3	0	6	11 (2c)
35	A1	0	0	1	0	2	17
35	A2	0	0	4	0	4	12 (1ade) (1c)
35	B1	0	0	3	0	4	13 (1d) (1ce)
35	B2	0	0	3	0	6	11 (2ce)
36	A1	0	0	1	0	2	17
36	A2	0	0	4	0	4	12 (1ade) (1ce)
36	B1	0	0	3	0	4	13 (1d) (1ce)
36	B2	0	0	3	0	6	11 (2ce)
37	A1	0	0	1	0	2	17
37	A2	0	0	4	0	4	12 (1ade) (1ce)
37	B1	0	0	3	0	4	13 (1d) (1ce)
37	B2	0	0	3	0	6	11 (2ce)
38	A1	0	0	1	0	2	17
38	A2	0	0	4	0	4	12 (1ade) (1ce)
38	B1	0	0	3	0	4	13 (1d) (1ce)
38	B2	0	0	3	0	6	11 (2ce)
39	A1	0	0	1	0	2	17
39	A2	0	0	4	0	4	12 (1ade) (1ce)
39	B1	0	0	3	0	4	13 (1d) (1ce)
39	B2	0	0	3	0	6	11 (2ce)

Table 12
2.16 mg/L: Embryo Survival and Hatching Data for the 90-Day Rainbow Trout,
Oncorhynchus mykiss, Early Life-Stage Toxicity Test (Continued)

Day	Replicate [†]	Dead Eggs Removed	Dead Larvae Removed	Cumulative Dead Eggs	Live Eggs Left	Cumulative Dead Larvae	Live Larvae
40	A1	0	0	1	0	2	17 (1ce) (1g)
40	A2	0	0	4	0	4	12 (1ade) (1ce)
40	B1	0	0	3	0	4	13 (1ce)
40	B2	0	0	3	0	6	11 (2ce)
41	A1	0	0	1	0	2	17 (1ceh) (1gf)
41	A2	0	0	4	0	4	12 (1ah) (1ceh)
41	B1	0	0	3	0	4	13 (1ceh)
41	B2	0	0	3	0	6	11 (2ce)
42	A1	0	0	1	0	2	17 (1ceh) (1fgi)
42	A2	0	0	4	0	4	12 (1afhi) (1ceh)
42	B1	0	0	3	0	4	13 (1ceh)
42	B2	0	0	3	0	6	11 (2cefh)
43	A1	0	1	1	0	3	16 (1fgi)
43	A2	0	0	4	0	4	12 (1afhi) (1ceh)
43	B1	0	0	3	0	4	13 (1ceh)
43	B2	0	0	3	0	6	11 (2cefi)
44	A1	0	0	1	0	3	16 (1fgi)
44	A2	0	0	4	0	4	12 (1efhi) (1ceh)
44	B1	0	0	3	0	4	13 (2cef)
44	B2	0	0	3	0	6	11 (1cefi)
45	A1	0	0	1	0	3	16 (1fgi)
45	A2	0	1	4	0	5	11
45	B1	0	1	3	0	5	12 (1cef)
45	B2	0	0	3	0	6	11 (1cefi)

Table 12
2.16 mg/L: Embryo Survival and Hatching Data for the 90-Day Rainbow Trout,
Oncorhynchus mykiss, Early Life-Stage Toxicity Test (Continued)

Day	Replicate [†]	Dead Eggs Removed	Dead Larvae Removed	Cumulative Dead Eggs	Live Eggs Left	Cumulative Dead Larvae	Live Larvae
46	A1	0	0	1	0	3	16 (1fgi)
46	A2	0	0	4	0	5	11
46	B1	0	0	3	0	5	12 (1cef)
46	B2	0	0	3	0	6	11 (1cefi)

[†] Four replicates per concentration (i.e., A1 - B2).

- a small in size (when compared to appropriate control(s))
- b scoliosis (includes: curved spine, corkscrew tail, and bent spine/tail)
- c mouth - potential jaw deformity
- d pale in color
- e lethargic
- f labored respiration (gasping)
- g loss of equilibrium
- h yolk sac present (not absorbed)
- i lying on bottom of test chamber

Table 13
4.66 mg/L: Embryo Survival and Hatching Data for the 90-Day Rainbow Trout,
Oncorhynchus mykiss, Early Life-Stage Toxicity Test

Day	Replicate [†]	Dead Eggs Removed	Dead Larvae Removed	Cumulative Dead Eggs	Live Eggs Left	Cumulative Dead Larvae	Live Larvae
0	A1	0	0	0	20	0	0
0	A2	0	0	0	20	0	0
0	B1	0	0	0	20	0	0
0	B2	0	0	0	20	0	0
1	A1	0	0	0	20	0	0
1	A2	0	0	0	20	0	0
1	B1	0	0	0	20	0	0
1	B2	0	0	0	20	0	0
2	A1	0	0	0	20	0	0
2	A2	0	0	0	20	0	0
2	B1	0	0	0	20	0	0
2	B2	0	0	0	20	0	0
3	A1	0	0	0	20	0	0
3	A2	0	0	0	20	0	0
3	B1	0	0	0	20	0	0
3	B2	0	0	0	20	0	0
4	A1	0	0	0	20	0	0
4	A2	0	0	0	20	0	0
4	B1	0	0	0	20	0	0
4	B2	0	0	0	20	0	0
5	A1	0	0	0	20	0	0
5	A2	0	0	0	20	0	0
5	B1	1	0	1	19	0	0
5	B2	0	0	0	20	0	0
6	A1	0	0	0	20	0	0
6	A2	0	0	0	20	0	0
6	B1	0	0	1	19	0	0
6	B2	0	0	0	20	0	0
7	A1	0	0	0	20	0	0
7	A2	0	0	0	20	0	0
7	B1	0	0	1	19	0	0
7	B2	0	0	0	20	0	0

Table 13
4.66 mg/L: Embryo Survival and Hatching Data for the 90-Day Rainbow Trout,
Oncorhynchus mykiss, Early Life-Stage Toxicity Test (Continued)

Day	Replicate [†]	Dead Eggs Removed	Dead Larvae Removed	Cumulative Dead Eggs	Live Eggs Left	Cumulative Dead Larvae	Live Larvae
8	A1	0	0	0	20	0	0
8	A2	0	0	0	20	0	0
8	B1	0	0	1	19	0	0
8	B2	0	0	0	20	0	0
9	A1	0	0	0	20	0	0
9	A2	0	0	0	20	0	0
9	B1	0	0	1	19	0	0
9	B2	0	0	0	20	0	0
10	A1	0	0	0	20	0	0
10	A2	0	0	0	20	0	0
10	B1	0	0	1	19	0	0
10	B2	0	0	0	20	0	0
11	A1	0	0	0	20	0	0
11	A2	0	0	0	20	0	0
11	B1	0	0	1	19	0	0
11	B2	1	0	1	19	0	0
12	A1	0	0	0	20	0	0
12	A2	0	0	0	20	0	0
12	B1	0	0	1	19	0	0
12	B2	0	0	1	19	0	0
13	A1	0	0	0	20	0	0
13	A2	0	0	0	20	0	0
13	B1	0	0	1	19	0	0
13	B2	0	0	1	19	0	0
14	A1	0	0	0	20	0	0
14	A2	0	0	0	20	0	0
14	B1	0	0	1	19	0	0
14	B2	0	0	1	19	0	0
15	A1	0	0	0	20	0	0
15	A2	0	0	0	20	0	0
15	B1	0	0	1	19	0	0
15	B2	3	0	4	16	0	0

Table 13
4.66 mg/L: Embryo Survival and Hatching Data for the 90-Day Rainbow Trout,
Oncorhynchus mykiss, Early Life-Stage Toxicity Test (Continued)

Day	Replicate [†]	Dead Eggs Removed	Dead Larvae Removed	Cumulative Dead Eggs	Live Eggs Left	Cumulative Dead Larvae	Live Larvae
16	A1	0	0	0	20	0	0
16	A2	0	0	0	20	0	0
16	B1	0	0	1	19	0	0
16	B2	0	0	4	16	0	0
17	A1	1	0	1	19	0	0
17	A2	0	0	0	20	0	0
17	B1	0	0	1	19	0	0
17	B2	0	0	4	16	0	0
18	A1	0	0	1	19	0	0
18	A2	0	0	0	20	0	0
18	B1	0	0	1	19	0	0
18	B2	0	0	4	16	0	0
19	A1	0	0	1	19	0	0
19	A2	1	0	1	19	0	0
19	B1	0	0	1	19	0	0
19	B2	0	0	4	16	0	0
20	A1	0	0	1	19	0	0
20	A2	0	0	1	19	0	0
20	B1	0	0	1	16	0	3
20	B2	0	0	4	16	0	0
21	A1	0	1	1	15	1	3
21	A2	0	0	1	14	0	5
21	B1	0	0	1	12	0	7
21	B2	0	0	4	14	0	2
22	A1	0	0	1	7	1	11
22	A2	0	0	1	6	0	13
22	B1	1	0	2	2	0	16
22	B2	0	0	4	5	0	11
23	A1	1	1	2	0	2	16
23	A2	3	3	4	0	3	13
23	B1	1	2	3	0	2	15
23	B2	1	0	5	0	0	15

Table 13
4.66 mg/L: Embryo Survival and Hatching Data for the 90-Day Rainbow Trout,
Oncorhynchus mykiss, Early Life-Stage Toxicity Test (Continued)

Day	Replicate [†]	Dead Eggs Removed	Dead Larvae Removed	Cumulative Dead Eggs	Live Eggs Left	Cumulative Dead Larvae	Live Larvae
24	A1	0	1	2	0	3	15
24	A2	0	0	4	0	3	13
24	B1	0	1	3	0	3	14
24	B2	0	3	5	0	3	12
25	A1	0	1	2	0	4	14
25	A2	0	1	4	0	4	12
25	B1	0	0	3	0	3	14
25	B2	0	0	5	0	3	12
26	A1	0	1	2	0	5	13
26	A2	0	0	4	0	4	12
26	B1	0	1	3	0	4	13
26	B2	0	1	5	0	4	11
27	A1	0	0	2	0	5	13
27	A2	0	0	4	0	4	12
27	B1	0	0	3	0	4	13
27	B2	0	0	5	0	4	11
28	A1	0	0	2	0	5	13
28	A2	0	0	4	0	4	12
28	B1	0	0	3	0	4	13
28	B2	0	0	5	0	4	11
29	A1	0	0	2	0	5	13
29	A2	0	0	4	0	4	12
29	B1	0	0	3	0	4	13
29	B2	0	0	5	0	4	11
30	A1	0	0	2	0	5	13 (1c)
30	A2	0	0	4	0	4	12
30	B1	0	0	3	0	4	13
30	B2	0	0	5	0	4	11
31	A1	0	0	2	0	5	13
31	A2	0	0	4	0	4	12
31	B1	0	0	3	0	4	13
31	B2	0	0	5	0	4	11

Table 13
4.66 mg/L: Embryo Survival and Hatching Data for the 90-Day Rainbow Trout,
Oncorhynchus mykiss, Early Life-Stage Toxicity Test (Continued)

Day	Replicate [†]	Dead Eggs Removed	Dead Larvae Removed	Cumulative Dead Eggs	Live Eggs Left	Cumulative Dead Larvae	Live Larvae
32	A1	0	0	2	0	5	13
32	A2	0	0	4	0	4	12
32	B1	0	0	3	0	4	13 (1b)
32	B2	0	0	5	0	4	11 (1a)
33	A1	0	0	2	0	5	13
33	A2	0	0	4	0	4	12 (1b)
33	B1	0	0	3	0	4	13 (1b)
33	B2	0	0	5	0	4	11 (1a)
34	A1	0	0	2	0	5	13 (1cd)
34	A2	0	0	4	0	4	12 (1b)
34	B1	0	1	3	0	5	12 (1bc) (1c)
34	B2	0	0	5	0	4	11 (2c) (1cd)
35	A1	0	0	2	0	5	13 (1d)
35	A2	0	0	4	0	4	12 (1ab) (1bcd)
35	B1	0	0	3	0	5	12 (1cd) (1d)
35	B2	0	0	5	0	4	11 (2cd)
36	A1	0	0	2	0	5	13 (1cd)
36	A2	0	0	4	0	4	12 (1ab) (1bcd)
36	B1	0	0	3	0	5	12 (1cd) (1d)
36	B2	0	0	5	0	4	11 (2cd)
37	A1	0	0	2	0	5	13 (1cd)
37	A2	0	0	4	0	4	12 (1ab) (1bcd)
37	B1	0	0	3	0	5	12 (1cd) (1d)
37	B2	0	0	5	0	4	11 (2cd)
38	A1	0	0	2	0	5	13 (1cd)
38	A2	0	0	4	0	4	12 (1abd) (1c) (1bcd)
38	B1	0	0	3	0	5	12 (1cd) (1d)
38	B2	0	0	5	0	4	11 (1acd) (1cd)
39	A1	0	0	2	0	5	13 (1cd)
39	A2	0	0	4	0	4	12 (1abd) (1c) (1bcd)
39	B1	0	0	3	0	5	12 (1cd) (1d)
39	B2	0	0	5	0	4	11 (1acd) (1cd)

Table 13
4.66 mg/L: Embryo Survival and Hatching Data for the 90-Day Rainbow Trout,
Oncorhynchus mykiss, Early Life-Stage Toxicity Test (Continued)

Day	Replicate [†]	Dead Eggs Removed	Dead Larvae Removed	Cumulative Dead Eggs	Live Eggs Left	Cumulative Dead Larvae	Live Larvae
40	A1	0	0	2	0	5	13 (1cd)
40	A2	0	0	4	0	4	12 (1abd)
40	B1	0	1	3	0	6	11 (1c)
40	B2	0	0	5	0	4	11 (1bcde)
41	A1	0	0	2	0	5	13 (1d)
41	A2	0	0	4	0	4	12 (1acd)
41	B1	0	0	3	0	6	11 (1cd)
41	B2	0	0	5	0	4	11 (1cdf)
42	A1	0	1	2	0	6	12 (1abd)
42	A2	0	0	4	0	4	12 (1c)
42	B1	0	0	3	0	6	11 (1bcdef)
42	B2	0	0	5	0	4	11 (1d)
43	A1	0	0	2	0	6	12 (1acdf)
43	A2	0	0	4	0	4	12 (1cdf)
43	B1	0	0	3	0	6	11 (1bcdef)
43	B2	0	0	5	0	4	11 (1acdf)
44	A1	0	0	2	0	6	12 (1cdf)
44	A2	0	0	4	0	4	12 (1bcdef)
44	B1	0	0	3	0	6	11 (1bcdef)
44	B2	0	0	5	0	4	11 (1acdf)
45	A1	0	0	2	0	6	12 (1cdf)
45	A2	0	1	4	0	5	11
45	B1	0	1	3	0	7	10
45	B2	0	2	5	0	6	9

Table 13
4.66 mg/L: Embryo Survival and Hatching Data for the 90-Day Rainbow Trout,
Oncorhynchus mykiss, Early Life-Stage Toxicity Test (Continued)

Day	Replicate [†]	Dead Eggs Removed	Dead Larvae Removed	Cumulative Dead Eggs	Live Eggs Left	Cumulative Dead Larvae	Live Larvae
46	A1	0	0	2	0	6	12
46	A2	0	0	4	0	5	11
46	B1	0	0	3	0	7	10
46	B2	0	0	5	0	6	9

[†] Four replicates per concentration (i.e., A1 - B2).

- a small in size (when compared to appropriate control(s))
- b scoliosis (includes: curved spine, corkscrew tail, and bent spine/tail)
- c mouth - potential jaw deformity
- d lethargic
- e labored respiration (gasping)
- f yolk sac present (not absorbed)

Table 14
8.89 mg/L: Embryo Survival and Hatching Data for the 90-Day Rainbow Trout,
Oncorhynchus mykiss, Early Life-Stage Toxicity Test

Day	Replicate [†]	Dead Eggs Removed	Dead Larvae Removed	Cumulative Dead Eggs	Live Eggs Left	Cumulative Dead Larvae	Live Larvae
0	A1	0	0	0	20	0	0
0	A2	0	0	0	20	0	0
0	B1	0	0	0	20	0	0
0	B2	0	0	0	20	0	0
1	A1	0	0	0	20	0	0
1	A2	0	0	0	20	0	0
1	B1	0	0	0	20	0	0
1	B2	0	0	0	20	0	0
2	A1	0	0	0	20	0	0
2	A2	0	0	0	20	0	0
2	B1	0	0	0	20	0	0
2	B2	0	0	0	20	0	0
3	A1	0	0	0	20	0	0
3	A2	0	0	0	20	0	0
3	B1	0	0	0	20	0	0
3	B2	0	0	0	20	0	0
4	A1	0	0	0	20	0	0
4	A2	0	0	0	20	0	0
4	B1	0	0	0	20	0	0
4	B2	0	0	0	20	0	0
5	A1	0	0	0	20	0	0
5	A2	0	0	0	20	0	0
5	B1	0	0	0	20	0	0
5	B2	0	0	0	20	0	0
6	A1	0	0	0	20	0	0
6	A2	1	0	1	19	0	0
6	B1	0	0	0	20	0	0
6	B2	0	0	0	20	0	0
7	A1	0	0	0	20	0	0
7	A2	0	0	1	19	0	0
7	B1	0	0	0	20	0	0
7	B2	0	0	0	20	0	0

Table 14
8.89 mg/L: Embryo Survival and Hatching Data for the 90-Day Rainbow Trout,
Oncorhynchus mykiss, Early Life-Stage Toxicity Test (Continued)

Day	Replicate [†]	Dead Eggs Removed	Dead Larvae Removed	Cumulative Dead Eggs	Live Eggs Left	Cumulative Dead Larvae	Live Larvae
8	A1	0	0	0	20	0	0
8	A2	0	0	1	19	0	0
8	B1	0	0	0	20	0	0
8	B2	0	0	0	20	0	0
9	A1	0	0	0	20	0	0
9	A2	0	0	1	19	0	0
9	B1	0	0	0	20	0	0
9	B2	0	0	0	20	0	0
10	A1	0	0	0	20	0	0
10	A2	0	0	1	19	0	0
10	B1	0	0	0	20	0	0
10	B2	0	0	0	20	0	0
11	A1	0	0	0	20	0	0
11	A2	0	0	1	19	0	0
11	B1	0	0	0	20	0	0
11	B2	0	0	0	20	0	0
12	A1	0	0	0	20	0	0
12	A2	0	0	1	19	0	0
12	B1	1	0	1	19	0	0
12	B2	1	0	1	19	0	0
13	A1	0	0	0	20	0	0
13	A2	0	0	1	19	0	0
13	B1	0	0	1	19	0	0
13	B2	0	0	1	19	0	0
14	A1	0	0	0	20	0	0
14	A2	1	0	2	18	0	0
14	B1	0	0	1	19	0	0
14	B2	0	0	1	19	0	0
15	A1	0	0	0	20	0	0
15	A2	0	0	2	18	0	0
15	B1	0	0	1	19	0	0
15	B2	0	0	1	19	0	0

Table 14
8.89 mg/L: Embryo Survival and Hatching Data for the 90-Day Rainbow Trout,
Oncorhynchus mykiss, Early Life-Stage Toxicity Test (Continued)

Day	Replicate [†]	Dead Eggs Removed	Dead Larvae Removed	Cumulative Dead Eggs	Live Eggs Left	Cumulative Dead Larvae	Live Larvae
16	A1	0	0	0	20	0	0
16	A2	0	0	2	18	0	0
16	B1	0	0	1	19	0	0
16	B2	0	0	1	19	0	0
17	A1	0	0	0	20	0	0
17	A2	0	0	2	18	0	0
17	B1	1	0	2	18	0	0
17	B2	0	0	1	19	0	0
18	A1	0	0	0	20	0	0
18	A2	0	0	2	18	0	0
18	B1	0	0	2	18	0	0
18	B2	0	0	1	19	0	0
19	A1	0	0	0	20	0	0
19	A2	0	0	2	18	0	0
19	B1	0	0	2	18	0	0
19	B2	0	0	1	19	0	0
20	A1	0	0	0	20	0	0
20	A2	0	0	2	18	0	0
20	B1	0	0	2	17	0	1
20	B2	0	0	1	19	0	0
21	A1	0	0	0	15	0	5
21	A2	0	0	2	16	0	2
21	B1	0	0	2	15	0	3
21	B2	0	1	1	15	1	3
22	A1	0	0	0	6	0	14
22	A2	0	0	2	9	0	9
22	B1	1	0	3	8	0	9
22	B2	0	0	1	9	1	9
23	A1	4	0	4	0	0	16
23	A2	2	2	4	0	2	14
23	B1	1	0	4	0	0	16
23	B2	1	3	2	0	4	14

Table 14
8.89 mg/L: Embryo Survival and Hatching Data for the 90-Day Rainbow Trout,
Oncorhynchus mykiss, Early Life-Stage Toxicity Test (Continued)

Day	Replicate [†]	Dead Eggs Removed	Dead Larvae Removed	Cumulative Dead Eggs	Live Eggs Left	Cumulative Dead Larvae	Live Larvae
24	A1	0	1	4	0	1	15
24	A2	0	0	4	0	2	14
24	B1	0	2	4	0	2	14
24	B2	0	1	2	0	5	13
25	A1	0	0	4	0	1	15
25	A2	0	0	4	0	2	14
25	B1	0	0	4	0	2	14
25	B2	0	0	2	0	5	13
26	A1	0	2	4	0	3	13
26	A2	0	0	4	0	2	14
26	B1	0	0	4	0	2	14
26	B2	0	0	2	0	5	13
27	A1	0	2	4	0	3	13
27	A2	0	0	4	0	2	14
27	B1	0	1	4	0	3	13
27	B2	0	0	2	0	5	13
28	A1	0	0	4	0	3	13
28	A2	0	0	4	0	2	14
28	B1	0	0	4	0	3	13
28	B2	0	0	2	0	5	13
29	A1	0	0	4	0	3	13
29	A2	0	0	4	0	2	14
29	B1	0	0	4	0	3	13
29	B2	0	0	2	0	5	13
30	A1	0	0	4	0	3	13
30	A2	0	0	4	0	2	14 (1c)
30	B1	0	0	4	0	3	13
30	B2	0	0	2	0	5	13
31	A1	0	0	4	0	3	13
31	A2	0	0	4	0	2	14
31	B1	0	0	4	0	3	13 (1a)
31	B2	0	0	2	0	5	13

Table 14
8.89 mg/L: Embryo Survival and Hatching Data for the 90-Day Rainbow Trout,
Oncorhynchus mykiss, Early Life-Stage Toxicity Test (Continued)

Day	Replicate [†]	Dead Eggs Removed	Dead Larvae Removed	Cumulative Dead Eggs	Live Eggs Left	Cumulative Dead Larvae	Live Larvae	
32	A1	0	0	4	0	3	13	
32	A2	0	0	4	0	2	14	(1c)
32	B1	0	0	4	0	3	13	(1a)
32	B2	0	0	2	0	5	13	
33	A1	0	0	4	0	3	13	
33	A2	0	0	4	0	2	14	
33	B1	0	0	4	0	3	13	(1a)
33	B2	0	0	2	0	5	13	(1b)
34	A1	0	0	4	0	3	13	
34	A2	0	0	4	0	2	14	(2ce)
34	B1	0	0	4	0	3	13	(1ae)
34	B2	0	0	2	0	5	13	(1b)
35	A1	0	0	4	0	3	13	(1d)
35	A2	0	0	4	0	2	14	(2ce)
35	B1	0	0	4	0	3	13	(1ae)
35	B2	0	0	2	0	5	13	(1be)
36	A1	0	0	4	0	3	13	
36	A2	0	0	4	0	2	14	(2ce)
36	B1	0	0	4	0	3	13	(1ae)
36	B2	0	0	2	0	5	13	(1bde)
37	A1	0	0	4	0	3	13	
37	A2	0	0	4	0	2	14	(2ce)
37	B1	0	0	4	0	3	13	(1ae)
37	B2	0	0	2	0	5	13	(1bde)
38	A1	0	0	4	0	3	13	
38	A2	0	0	4	0	2	14	(2ce)
38	B1	0	0	4	0	3	13	(1ae)
38	B2	0	0	2	0	5	13	(1bde)
39	A1	0	0	4	0	3	13	
39	A2	0	0	4	0	2	14	(2ce)
39	B1	0	0	4	0	3	13	(1ae)
39	B2	0	0	2	0	5	13	(1bde)

Table 14
8.89 mg/L: Embryo Survival and Hatching Data for the 90-Day Rainbow Trout,
Oncorhynchus mykiss, Early Life-Stage Toxicity Test (Continued)

Day	Replicate [†]	Dead Eggs Removed	Dead Larvae Removed	Cumulative Dead Eggs	Live Eggs Left	Cumulative Dead Larvae	Live Larvae	
40	A1	0	0	4	0	3	13	
40	A2	0	0	4	0	2	14	(2ce)
40	B1	0	0	4	0	3	13	(1ae)
40	B2	0	0	2	0	5	13	(1bde)
41	A1	0	0	4	0	3	13	
41	A2	0	0	4	0	2	14	(1ce)
41	B1	0	0	4	0	3	13	(1ceh)
41	B2	0	0	2	0	5	13	(1aeh)
42	A1	0	0	4	0	3	13	(1bde)
42	A2	0	1	4	0	3	13	
42	B1	0	1	4	0	4	12	
42	B2	0	0	2	0	5	13	(1bde)
43	A1	0	0	4	0	3	13	
43	A2	0	0	4	0	3	13	
43	B1	0	0	4	0	4	12	
43	B2	0	0	2	0	5	13	(1bdef)
44	A1	0	0	4	0	3	13	
44	A2	0	0	4	0	3	13	
44	B1	0	0	4	0	4	12	
44	B2	0	0	2	0	5	13	(1bdef)
45	A1	0	0	4	0	3	13	
45	A2	0	0	4	0	3	13	(1cfi)
45	B1	0	0	4	0	4	12	
45	B2	0	0	2	0	5	13	(1bdef)

Table 14
8.89 mg/L: Embryo Survival and Hatching Data for the 90-Day Rainbow Trout,
Oncorhynchus mykiss, Early Life-Stage Toxicity Test (Continued)

Day	Replicate [†]	Dead Eggs Removed	Dead Larvae Removed	Cumulative Dead Eggs	Live Eggs Left	Cumulative Dead Larvae	Live Larvae
46	A1	0	0	4	0	3	13
46	A2	0	0	4	0	3	13
46	B1	0	0	4	0	4	12
46	B2	0	0	2	0	5	13

[†] Four replicates per concentration (i.e., A1 - B2).

a small in size (when compared to appropriate control(s))

b scoliosis (includes: curved spine, corkscrew tail, and bent spine/tail)

c mouth - potential jaw deformity

d pale in color

e lethargic

f labored respiration (gasping)

g loss of equilibrium

h yolk sac present (not absorbed)

i lying on bottom of test chamber

Table 15
Dilution Water Control: Swim-Up Data for the 90-Day Rainbow Trout, *Oncorhynchus mykiss*,
Early Life-Stage Toxicity Test

Day	Replicate	Total Live Larvae	Total Larvae Swimming Up
35	A1	12	0
35	A2	15	1
35	B1	14	0
35	B2	14	0
36	A1	12	1
36	A2	15	1
36	B1	14	0
36	B2	14	0
37	A1	12	3
37	A2	15	2
37	B1	14	2
37	B2	14	1
38	A1	11	4
38	A2	15	2
38	B1	14	4
38	B2	14	3
39	A1	11	4
39	A2	14	4
39	B1	13	4
39	B2	14	3
40	A1	11	7
40	A2	14	5
40	B1	13	5
40	B2	14	5
41	A1	11	7
41	A2	14	5
41	B1	13	5
41	B2	14	5
42	A1	10	7
42	A2	14	6
42	B1	13	6
42	B2	14	6

Table 15
Dilution Water Control: Swim-Up Data for the 90-Day Rainbow Trout, *Oncorhynchus mykiss*,
Early Life-Stage Toxicity Test (Continued)

Day	Replicate	Total Live Larvae	Total Larvae Swimming Up
43	A1	10	9
43	A2	14	11
43	B1	13	11
43	B2	14	12
44	A1	10	9
44	A2	14	13
44	B1	13	13
44	B2	14	12
45	A1	10	9
45	A2	14	14
45	B1	13	13
45	B2	13	12
46	A1	10	9
46	A2	14	14
46	B1	13	13
46	B2	13	12

Table 16
0.651 mg/L: Swim-Up Data for the 90-Day Rainbow Trout, *Oncorhynchus mykiss*, Early
Life-Stage Toxicity Test

Day	Replicate	Total Live Larvae	Total Larvae Swimming Up
35	A1	10	0
35	A2	14	0
35	B1	15	0
35	B2	13	0
36	A1	10	0
36	A2	14	0
36	B1	15	1
36	B2	13	0
37	A1	10	0
37	A2	14	2
37	B1	15	1
37	B2	13	0
38	A1	9	0
38	A2	14	2
38	B1	15	3
38	B2	13	1
39	A1	9	1
39	A2	14	4
39	B1	15	4
39	B2	13	2
40	A1	9	4
40	A2	13	5
40	B1	15	4
40	B2	13	2
41	A1	9	4
41	A2	13	5
41	B1	15	4
41	B2	13	2
42	A1	9	5
42	A2	13	6
42	B1	15	6
42	B2	12	5

Table 16
0.651 mg/L: Swim-Up Data for the 90-Day Rainbow Trout, *Oncorhynchus mykiss*, Early
Life-Stage Toxicity Test (Continued)

Day	Replicate	Total Live Larvae	Total Larvae Swimming Up
43	A1	9	8
43	A2	13	10
43	B1	15	9
43	B2	12	8
44	A1	9	8
44	A2	13	12
44	B1	15	13
44	B2	12	10
45	A1	9	8
45	A2	13	12
45	B1	15	13
45	B2	11	10
46	A1	9	8
46	A2	13	12
46	B1	14	13
46	B2	10	10

Table 17
1.08 mg/L: Swim-Up Data for the 90-Day Rainbow Trout, *Oncorhynchus mykiss*, Early
Life-Stage Toxicity Test

Day	Replicate	Total Live Larvae	Total Larvae Swimming Up
35	A1	14	0
35	A2	14	0
35	B1	14	0
35	B2	10	0
36	A1	14	3
36	A2	14	0
36	B1	14	0
36	B2	10	0
37	A1	14	3
37	A2	14	2
37	B1	14	1
37	B2	10	1
38	A1	13	4
38	A2	13	4
38	B1	14	4
38	B2	10	1
39	A1	13	4
39	A2	12	4
39	B1	14	4
39	B2	10	2
40	A1	13	5
40	A2	12	4
40	B1	14	5
40	B2	10	3
41	A1	13	5
41	A2	12	4
41	B1	14	5
41	B2	10	3
42	A1	12	6
42	A2	12	6
42	B1	14	6
42	B2	10	5

Table 17
1.08 mg/L: Swim-Up Data for the 90-Day Rainbow Trout, *Oncorhynchus mykiss*, Early
Life-Stage Toxicity Test (Continued)

Day	Replicate	Total Live Larvae	Total Larvae Swimming Up
43	A1	12	9
43	A2	12	10
43	B1	14	9
43	B2	10	8
44	A1	12	12
44	A2	12	11
44	B1	14	13
44	B2	10	9
45	A1	12	12
45	A2	12	12
45	B1	14	13
45	B2	9	9
46	A1	12	12
46	A2	12	12
46	B1	14	13
46	B2	9	9

Table 18
2.16 mg/L: Swim-Up Data for the 90-Day Rainbow Trout, *Oncorhynchus mykiss*, Early
Life-Stage Toxicity Test

Day	Replicate	Total Live Larvae	Total Larvae Swimming Up
35	A1	17	0
35	A2	12	0
35	B1	13	0
35	B2	11	1
36	A1	17	0
36	A2	12	0
36	B1	13	1
36	B2	11	1
37	A1	17	1
37	A2	12	0
37	B1	13	2
37	B2	11	1
38	A1	17	2
38	A2	12	3
38	B1	13	2
38	B2	11	2
39	A1	17	4
39	A2	12	3
39	B1	13	3
39	B2	11	2
40	A1	17	6
40	A2	12	3
40	B1	13	5
40	B2	11	3
41	A1	17	6
41	A2	12	3
41	B1	13	5
41	B2	11	3
42	A1	17	7
42	A2	12	7
42	B1	13	7
42	B2	11	5

Table 18
2.16 mg/L: Swim-Up Data for the 90-Day Rainbow Trout, *Oncorhynchus mykiss*, Early
Life-Stage Toxicity Test (Continued)

Day	Replicate	Total Live Larvae	Total Larvae Swimming Up
43	A1	16	11
43	A2	12	9
43	B1	13	11
43	B2	11	8
44	A1	16	15
44	A2	12	11
44	B1	13	9
44	B2	11	11
45	A1	16	15
45	A2	11	11
45	B1	12	11
45	B2	11	11
46	A1	16	15
46	A2	11	11
46	B1	12	11
46	B2	11	10

Table 19
4.66 mg/L: Swim-Up Data for the 90-Day Rainbow Trout, *Oncorhynchus mykiss*, Early
Life-Stage Toxicity Test

Day	Replicate	Total Live Larvae	Total Larvae Swimming Up
35	A1	13	0
35	A2	12	0
35	B1	12	0
35	B2	11	0
36	A1	13	0
36	A2	12	0
36	B1	12	0
36	B2	11	1
37	A1	13	0
37	A2	12	2
37	B1	12	0
37	B2	11	1
38	A1	13	1
38	A2	12	4
38	B1	12	1
38	B2	11	1
39	A1	13	4
39	A2	12	4
39	B1	12	3
39	B2	11	3
40	A1	13	5
40	A2	12	5
40	B1	11	3
40	B2	11	3
41	A1	13	5
41	A2	12	5
41	B1	11	3
41	B2	11	3
42	A1	12	6
42	A2	12	6
42	B1	11	5
42	B2	11	5

Table 19
4.66 mg/L: Swim-Up Data for the 90-Day Rainbow Trout, *Oncorhynchus mykiss*, Early
Life-Stage Toxicity Test (Continued)

Day	Replicate	Total Live Larvae	Total Larvae Swimming Up
43	A1	12	9
43	A2	12	10
43	B1	11	9
43	B2	11	8
44	A1	12	12
44	A2	12	11
44	B1	11	10
44	B2	11	9
45	A1	12	12
45	A2	11	11
45	B1	10	10
45	B2	9	9
46	A1	12	12
46	A2	11	11
46	B1	10	10
46	B2	9	9

Table 20
8.89 mg/L: Swim-Up Data for the 90-Day Rainbow Trout, *Oncorhynchus mykiss*, Early
Life-Stage Toxicity Test

Day	Replicate	Total Live Larvae	Total Larvae Swimming Up
35	A1	13	0
35	A2	14	0
35	B1	13	0
35	B2	13	0
36	A1	13	1
36	A2	14	1
36	B1	13	2
36	B2	13	1
37	A1	13	1
37	A2	14	2
37	B1	13	4
37	B2	13	3
38	A1	13	2
38	A2	14	2
38	B1	13	5
38	B2	13	3
39	A1	13	3
39	A2	14	4
39	B1	13	5
39	B2	13	4
40	A1	13	5
40	A2	14	5
40	B1	13	5
40	B2	13	5
41	A1	13	5
41	A2	14	5
41	B1	13	7
41	B2	13	5
42	A1	13	6
42	A2	13	7
42	B1	12	7
42	B2	13	6

Table 20
8.89 mg/L: Swim-Up Data for the 90-Day Rainbow Trout, *Oncorhynchus mykiss*, Early
Life-Stage Toxicity Test (Continued)

Day	Replicate	Total Live Larvae	Total Larvae Swimming Up
43	A1	13	10
43	A2	13	11
43	B1	12	11
43	B2	13	10
44	A1	13	12
44	A2	13	13
44	B1	12	12
44	B2	13	12
45	A1	13	13
45	A2	13	13
45	B1	12	12
45	B2	13	12
46	A1	13	13
46	A2	13	12
46	B1	12	12
46	B2	13	12

Table 21
Dilution Water Control: Larval Mortality After Thinning for the 90-Day Rainbow Trout,
Oncorhynchus mykiss, Early Life-Stage Toxicity Test

Day	Replicate	Larvae at Transfer	Dead Larvae Removed	Cumulative Dead Larvae	Live Larvae Remaining
46	A	15	0	0	15
46	B	15	0	0	15
47	A	15	0	0	15
47	B	15	0	0	15
48	A	15	0	0	15
48	B	15	0	0	15
49	A	15	0	0	15
49	B	15	0	0	15
50	A	15	0	0	15
50	B	15	0	0	15
51	A	15	0	0	15
51	B	15	0	0	15
52	A	15	0	0	15
52	B	15	0	0	15
53	A	15	0	0	15
53	B	15	0	0	15
54	A	15	0	0	15
54	B	15	0	0	15
55	A	15	0	0	15
55	B	15	0	0	15 (lace)
56	A	15	0	0	15
56	B	15	0	0	15 (1abcd)
57	A	15	0	0	15 (1a)
57	B	15	0	0	15 (1abcd)
58	A	15	0	0	15 (1a)
58	B	15	0	0	15 (1abcdf)
59	A	15	0	0	15 (1a)
59	B	15	0	0	15 (1abcdf)
60	A	15	0	0	15 (1a)
60	B	15	0	0	15 (1abcdf)
61	A	15	0	0	15 (1a)
61	B	15	0	0	15 (1abcdf)
62	A	15	0	0	15 (1a)
62	B	15	0	0	15 (1abcdf)

Table 21
Dilution Water Control: Larval Mortality After Thinning for the 90-Day Rainbow Trout,
Oncorhynchus mykiss, Early Life-Stage Toxicity Test (Continued)

Day	Replicate	Larvae at Transfer	Dead Larvae Removed	Cumulative Dead Larvae	Live Larvae Remaining
63	A	15	0	0	15 (1a)
63	B	15	0	0	15 (1abcdf)
64	A	15	0	0	15 (1a)
64	B	15	0	0	15 (1abcdf)
65	A	15	0	0	15 (1a)
65	B	15	0	0	15 (1abcdf)
66	A	15	0	0	15 (1a)
66	B	15	0	0	15 (1abcdf)
67	A	15	0	0	15 (1a)
67	B	15	0	0	15 (1abcdf)
68	A	15	0	0	15 (1a)
68	B	15	1	1	14
69	A	15	0	0	15 (1a)
69	B	15	0	1	14
70	A	15	0	0	15 (1a)
70	B	15	0	1	14 (1a)
71	A	15	0	0	15 (1a)
71	B	15	0	1	14 (1a)
72	A	15	0	0	15 (1a)
72	B	15	0	1	14 (1a)
73	A	15	0	0	15 (1a)
73	B	15	0	1	14 (1ad)
74	A	15	0	0	15 (1a)
74	B	15	0	1	14 (1ad)
75	A	15	0	0	15 (1a)
75	B	15	0	1	14 (1a)
76	A	15	0	0	15 (1a)
76	B	15	0	1	14 (1a)
77	A	15	0	0	15 (1a)
77	B	15	0	1	14 (1a)
78	A	15	0	0	15 (1a)
78	B	15	0	1	14 (1a)

Table 21
Dilution Water Control: Larval Mortality After Thinning for the 90-Day Rainbow Trout,
Oncorhynchus mykiss, Early Life-Stage Toxicity Test (Continued)

Day	Replicate	Larvae at Transfer	Dead Larvae Removed	Cumulative Dead Larvae	Live Larvae Remaining	
79	A	15	0	0	15	(1a)
79	B	15	0	1	14	(1a)
80	A	15	1	1	14	
80	B	15	0	1	14	(1a)
81	A	15	0	1	14	
81	B	15	0	1	14	(1a)
82	A	15	0	1	14	
82	B	15	0	1	14	(1a)
83	A	15	0	1	14	
83	B	15	0	1	14	(1a)
84	A	15	0	1	14	
84	B	15	0	1	14	(1a)
85	A	15	0	1	14	
85	B	15	0	1	14	(1a)
86	A	15	0	1	14	
86	B	15	0	1	14	(1a)
87	A	15	0	1	14	
87	B	15	0	1	14	(1a)
88	A	15	0	1	14	
88	B	15	0	1	14	(1a)
89	A	15	0	1	14	
89	B	15	0	1	14	(1a)
90	A	15	0	1	14	
90	B	15	0	1	14	(1a)

- a small in size (when compared to appropriate control(s))
- b lethargic
- c labored respiration (gasping)
- d lying on bottom of test chamber
- e erratic swimming
- f appears that other fish are biting/eating

Table 22
0.651 mg/L: Larval Mortality After Thinning for the 90-Day Rainbow Trout,
Oncorhynchus mykiss, Early Life-Stage Toxicity Test

Day	Replicate	Larvae at Transfer	Dead Larvae Removed	Cumulative Dead Larvae	Live Larvae Remaining
46	A	15	0	0	15
46	B	15	0	0	15
47	A	15	0	0	15
47	B	15	0	0	15
48	A	15	0	0	15
48	B	15	0	0	15
49	A	15	0	0	15
49	B	15	0	0	15
50	A	15	0	0	15
50	B	15	0	0	15
51	A	15	0	0	15
51	B	15	0	0	15
52	A	15	0	0	15
52	B	15	0	0	15
53	A	15	0	0	15
53	B	15	0	0	15
54	A	15	0	0	15
54	B	15	0	0	15
55	A	15	0	0	15
55	B	15	0	0	15
56	A	15	0	0	15 (1ab)
56	B	15	0	0	15
57	A	15	0	0	15 (1abcd)
57	B	15	0	0	15
58	A	15	0	0	15 (1abcde)
58	B	15	0	0	15
59	A	15	0	0	15 (1abcde)
59	B	15	0	0	15
60	A	15	0	0	15 (1abcde)
60	B	15	0	0	15
61	A	15	0	0	15 (1abcde)
61	B	15	0	0	15 (1ab)
62	A	15	1	1	14
62	B	15	0	0	15

Table 22
0.651 mg/L: Larval Mortality After Thinning for the 90-Day Rainbow Trout,
Oncorhynchus mykiss, Early Life-Stage Toxicity Test (Continued)

Day	Replicate	Larvae at Transfer	Dead Larvae Removed	Cumulative Dead Larvae	Live Larvae Remaining
63	A	15	0	1	14
63	B	15	0	0	15
64	A	15	0	1	14
64	B	15	0	0	15
65	A	15	0	1	14
65	B	15	0	0	15
66	A	15	0	1	14
66	B	15	0	0	15
67	A	15	0	1	14
67	B	15	0	0	15
68	A	15	0	1	14
68	B	15	0	0	15
69	A	15	0	1	14
69	B	15	0	0	15
70	A	15	0	1	14
70	B	15	0	0	15
71	A	15	0	1	14
71	B	15	0	0	15
72	A	15	0	1	14
72	B	15	0	0	15
73	A	15	0	1	14
73	B	15	0	0	15
74	A	15	0	1	14
74	B	15	0	0	15
75	A	15	0	1	14
75	B	15	0	0	15
76	A	15	0	1	14
76	B	15	0	0	15
77	A	15	0	1	14
77	B	15	0	0	15
78	A	15	0	1	14
78	B	15	0	0	15

Table 22
0.651 mg/L: Larval Mortality After Thinning for the 90-Day Rainbow Trout,
Oncorhynchus mykiss, Early Life-Stage Toxicity Test (Continued)

Day	Replicate	Larvae at Transfer	Dead Larvae Removed	Cumulative Dead Larvae	Live Larvae Remaining
79	A	15	0	1	14
79	B	15	0	0	15
80	A	15	0	1	14
80	B	15	0	0	15
81	A	15	0	1	14
81	B	15	0	0	15
82	A	15	0	1	14
82	B	15	0	0	15
83	A	15	0	1	14
83	B	15	0	0	15
84	A	15	0	1	14
84	B	15	0	0	15
85	A	15	0	1	14
85	B	15	0	0	15
86	A	15	0	1	14
86	B	15	0	0	15
87	A	15	0	1	14
87	B	15	0	0	15
88	A	15	0	1	14
88	B	15	0	0	15
89	A	15	0	1	14
89	B	15	0	0	15
90	A	15	0	1	14
90	B	15	0	0	15

- a small in size (when compared to appropriate control(s))
- b lethargic
- c labored respiration (gasping)
- d lying on bottom of test chamber
- e appears that other fish are biting/eating

Table 23
1.08 mg/L: Larval Mortality After Thinning for the 90-Day Rainbow Trout,
Oncorhynchus mykiss, Early Life-Stage Toxicity Test

Day	Replicate	Larvae at Transfer	Dead Larvae Removed	Cumulative Dead Larvae	Live Larvae Remaining	
46	A	15	0	0	15	
46	B	15	0	0	15	
47	A	15	0	0	15	
47	B	15	0	0	15	
48	A	15	0	0	15	
48	B	15	0	0	15	
49	A	15	0	0	15	
49	B	15	0	0	15	
50	A	15	0	0	15	
50	B	15	0	0	15	
51	A	15	0	0	15	
51	B	15	0	0	15	
52	A	15	0	0	15	
52	B	15	0	0	15	
53	A	15	0	0	15	
53	B	15	0	0	15	
54	A	15	0	0	15	
54	B	15	0	0	15	
55	A	15	0	0	15	
55	B	15	0	0	15	
56	A	15	0	0	15	
56	B	15	0	0	15	
57	A	15	0	0	15	
57	B	15	0	0	15	(1a)
58	A	15	0	0	15	
58	B	15	0	0	15	(1a)
59	A	15	0	0	15	
59	B	15	0	0	15	(1a)
60	A	15	0	0	15	
60	B	15	0	0	15	(1a)
61	A	15	0	0	15	
61	B	15	0	0	15	(1a)
62	A	15	0	0	15	
62	B	15	0	0	15	(1a)

Table 23
1.08 mg/L: Larval Mortality After Thinning for the 90-Day Rainbow Trout,
Oncorhynchus mykiss, Early Life-Stage Toxicity Test (Continued)

Day	Replicate	Larvae at Transfer	Dead Larvae Removed	Cumulative Dead Larvae	Live Larvae Remaining	
63	A	15	0	0	15	
63	B	15	0	0	15	(1a)
64	A	15	0	0	15	
64	B	15	0	0	15	(1a)
65	A	15	0	0	15	
65	B	15	0	0	15	(1a)
66	A	15	0	0	15	
66	B	15	0	0	15	(1adef)
67	A	15	0	0	15	
67	B	15	0	0	15	(1ade)
68	A	15	0	0	15	
68	B	15	0	0	15	(1ade)
69	A	15	0	0	15	
69	B	15	0	0	15	(1a)
70	A	15	0	0	15	
70	B	15	0	0	15	(1ab)
71	A	15	0	0	15	
71	B	15	0	0	15	(1ab)
72	A	15	0	0	15	
72	B	15	0	0	15	(1ab)
73	A	15	0	0	15	
73	B	15	0	0	15	(1abcf)
74	A	15	0	0	15	
74	B	15	0	0	15	(1abcf)
75	A	15	0	0	15	
75	B	15	0	0	15	(1abcf)
76	A	15	0	0	15	
76	B	15	0	0	15	(1abcf)
77	A	15	0	0	15	
77	B	15	0	0	15	(1abcf)
78	A	15	0	0	15	
78	B	15	0	0	15	(1abcf)

Table 23
1.08 mg/L: Larval Mortality After Thinning for the 90-Day Rainbow Trout,
Oncorhynchus mykiss, Early Life-Stage Toxicity Test (Continued)

Day	Replicate	Larvae at Transfer	Dead Larvae Removed	Cumulative Dead Larvae	Live Larvae Remaining	
79	A	15	0	0	15	
79	B	15	0	0	15	(1abcf)
80	A	15	0	0	15	
80	B	15	0	0	15	(1abcf)
81	A	15	0	0	15	
81	B	15	0	0	15	(1abcf)
82	A	15	0	0	15	
82	B	15	0	0	15	(1abcf)
83	A	15	0	0	15	
83	B	15	0	0	15	(1abcf)
84	A	15	0	0	15	
84	B	15	0	0	15	(1abcf)
85	A	15	0	0	15	
85	B	15	1	1	14	
86	A	15	0	0	15	
86	B	15	0	1	14	
87	A	15	0	0	15	
87	B	15	0	1	14	
88	A	15	0	0	15	
88	B	15	0	1	14	
89	A	15	0	0	15	
89	B	15	0	1	14	
90	A	15	0	0	15	
90	B	15	0	1	14	

- a small in size (when compared to appropriate control(s))
- b mouth - potential jaw deformity
- c lethargic
- d labored respiration (gasping)
- e loss of equilibrium
- f lying on bottom of test chamber

Table 24
2.16 mg/L: Larval Mortality After Thinning for the 90-Day Rainbow Trout,
Oncorhynchus mykiss, Early Life-Stage Toxicity Test

Day	Replicate	Larvae at Transfer	Dead Larvae Removed	Cumulative Dead Larvae	Live Larvae Remaining
46	A	15	0	0	15
46	B	15	0	0	15
47	A	15	0	0	15
47	B	15	0	0	15
48	A	15	0	0	15
48	B	15	0	0	15
49	A	15	0	0	15
49	B	15	0	0	15
50	A	15	0	0	15
50	B	15	0	0	15
51	A	15	0	0	15
51	B	15	0	0	15
52	A	15	0	0	15
52	B	15	0	0	15
53	A	15	0	0	15
53	B	15	0	0	15
54	A	15	0	0	15
54	B	15	0	0	15
55	A	15	0	0	15
55	B	15	0	0	15
56	A	15	0	0	15
56	B	15	0	0	15
57	A	15	0	0	15
57	B	15	0	0	15
58	A	15	0	0	15
58	B	15	0	0	15
59	A	15	0	0	15
59	B	15	0	0	15
60	A	15	0	0	15
60	B	15	0	0	15
61	A	15	0	0	15
61	B	15	0	0	15
62	A	15	0	0	15
62	B	15	0	0	15

Table 24
2.16 mg/L: Larval Mortality After Thinning for the 90-Day Rainbow Trout,
Oncorhynchus mykiss, Early Life-Stage Toxicity Test (Continued)

Day	Replicate	Larvae at Transfer	Dead Larvae Removed	Cumulative Dead Larvae	Live Larvae Remaining
63	A	15	0	0	15
63	B	15	0	0	15
64	A	15	0	0	15
64	B	15	0	0	15
65	A	15	0	0	15
65	B	15	0	0	15
66	A	15	0	0	15
66	B	15	0	0	15
67	A	15	0	0	15
67	B	15	0	0	15
68	A	15	0	0	15
68	B	15	0	0	15
69	A	15	0	0	15
69	B	15	0	0	15
70	A	15	0	0	15
70	B	15	0	0	15
71	A	15	0	0	15
71	B	15	0	0	15
72	A	15	0	0	15
72	B	15	0	0	15
73	A	15	0	0	15
73	B	15	0	0	15
74	A	15	0	0	15
74	B	15	0	0	15
75	A	15	0	0	15
75	B	15	0	0	15
76	A	15	0	0	15
76	B	15	0	0	15
77	A	15	0	0	15
77	B	15	0	0	15
78	A	15	0	0	15
78	B	15	0	0	15

(1a)

Table 24
2.16 mg/L: Larval Mortality After Thinning for the 90-Day Rainbow Trout,
Oncorhynchus mykiss, Early Life-Stage Toxicity Test (Continued)

Day	Replicate	Larvae at Transfer	Dead Larvae Removed	Cumulative Dead Larvae	Live Larvae Remaining	
79	A	15	0	0	15	(1a)
79	B	15	0	0	15	(2a)
80	A	15	0	0	15	
80	B	15	0	0	15	
81	A	15	0	0	15	
81	B	15	0	0	15	(1a)
82	A	15	0	0	15	(1a)
82	B	15	0	0	15	(1b)
83	A	15	0	0	15	
83	B	15	0	0	15	(1b)
84	A	15	0	0	15	
84	B	15	0	0	15	(1a)
85	A	15	0	0	15	
85	B	15	0	0	15	
86	A	15	0	0	15	
86	B	15	0	0	15	
87	A	15	0	0	15	
87	B	15	0	0	15	
88	A	15	0	0	15	
88	B	15	0	0	15	
89	A	15	0	0	15	
89	B	15	0	0	15	
90	A	15	0	0	15	
90	B	15	0	0	15	

- a left eye missing
b display of aggressive behavior

Table 25
4.66 mg/L: Larval Mortality After Thinning for the 90-Day Rainbow Trout,
Oncorhynchus mykiss, Early Life-Stage Toxicity Test

Day	Replicate	Larvae at Transfer	Dead Larvae Removed	Cumulative Dead Larvae	Live Larvae Remaining	
46	A	15	0	0	15	
46	B	15	0	0	15	
47	A	15	0	0	15	
47	B	15	0	0	15	
48	A	15	0	0	15	
48	B	15	0	0	15	
49	A	15	0	0	15	
49	B	15	0	0	15	
50	A	15	0	0	15	
50	B	15	0	0	15	
51	A	15	0	0	15	
51	B	15	0	0	15	
52	A	15	0	0	15	
52	B	15	0	0	15	
53	A	15	0	0	15	
53	B	15	0	0	15	
54	A	15	0	0	15	
54	B	15	0	0	15	
55	A	15	0	0	15	
55	B	15	0	0	15	
56	A	15	0	0	15	
56	B	15	0	0	15	
57	A	15	0	0	15	(1ac)
57	B	15	0	0	15	
58	A	15	0	0	15	(1ac)
58	B	15	0	0	15	
59	A	15	0	0	15	(1ac)
59	B	15	0	0	15	
60	A	15	0	0	15	(1ac)
60	B	15	0	0	15	
61	A	15	0	0	15	(1ac)
61	B	15	0	0	15	
62	A	15	0	0	15	(1ac)
62	B	15	0	0	15	

Table 25
4.66 mg/L: Larval Mortality After Thinning for the 90-Day Rainbow Trout,
Oncorhynchus mykiss, Early Life-Stage Toxicity Test (Continued)

Day	Replicate	Larvae at Transfer	Dead Larvae Removed	Cumulative Dead Larvae	Live Larvae Remaining	
63	A	15	0	0	15	(1ac)
63	B	15	0	0	15	
64	A	15	0	0	15	(1ac)
64	B	15	0	0	15	
65	A	15	0	0	15	(1ac)
65	B	15	0	0	15	
66	A	15	0	0	15	(1ac)
66	B	15	0	0	15	
67	A	15	0	0	15	(1ac)
67	B	15	0	0	15	
68	A	15	0	0	15	(1c)
68	B	15	0	0	15	
69	A	15	0	0	15	(1c)
69	B	15	0	0	15	
70	A	15	0	0	15	(1c)
70	B	15	0	0	15	
71	A	15	0	0	15	(1c)
71	B	15	0	0	15	
72	A	15	0	0	15	(1c)
72	B	15	0	0	15	
73	A	15	0	0	15	(1c)
73	B	15	0	0	15	
74	A	15	0	0	15	(1c)
74	B	15	0	0	15	
75	A	15	0	0	15	(1c)
75	B	15	0	0	15	
76	A	15	0	0	15	(1c)
76	B	15	0	0	15	
77	A	15	0	0	15	(1c)
77	B	15	0	0	15	
78	A	15	0	0	15	(1c,1d)
78	B	15	0	0	15	

Table 25
4.66 mg/L: Larval Mortality After Thinning for the 90-Day Rainbow Trout,
Oncorhynchus mykiss, Early Life-Stage Toxicity Test (Continued)

Day	Replicate	Larvae at Transfer	Dead Larvae Removed	Cumulative Dead Larvae	Live Larvae Remaining	
79	A	15	0	0	15	(1c,1d)
79	B	15	0	0	15	(1d,1d)
80	A	15	0	0	15	(1c,1d)
80	B	15	0	0	15	
81	A	15	0	0	15	(1c)
81	B	15	0	0	15	(1d)
82	A	15	0	0	15	(1d)
82	B	15	0	0	15	(1b)
83	A	15	0	0	15	
83	B	15	0	0	15	(1b)
84	A	15	0	0	15	(1c,1d)
84	B	15	0	0	15	
85	A	15	0	0	15	(1c)
85	B	15	0	0	15	
86	A	15	0	0	15	(1c)
86	B	15	0	0	15	
87	A	15	0	0	15	(1c)
87	B	15	0	0	15	
88	A	15	0	0	15	(1c)
88	B	15	0	0	15	
89	A	15	0	0	15	(1c)
89	B	15	0	0	15	
90	A	15	0	0	15	(1c)
90	B	15	0	0	15	

- a small in size (when compared to appropriate control(s))
- b pale in color
- c left eye missing
- d display of aggressive behavior

Table 26
8.89 mg/L: Larval Mortality After Thinning for the 90-Day Rainbow Trout,
Oncorhynchus mykiss, Early Life-Stage Toxicity Test

Day	Replicate	Larvae at Transfer	Dead Larvae Removed	Cumulative Dead Larvae	Live Larvae Remaining
46	A	15	0	0	15
46	B	15	0	0	15
47	A	15	0	0	15
47	B	15	0	0	15
48	A	15	0	0	15
48	B	15	0	0	15
49	A	15	0	0	15
49	B	15	0	0	15
50	A	15	0	0	15
50	B	15	0	0	15
51	A	15	0	0	15
51	B	15	0	0	15
52	A	15	0	0	15
52	B	15	0	0	15
53	A	15	0	0	15
53	B	15	0	0	15
54	A	15	0	0	15
54	B	15	0	0	15
55	A	15	0	0	15
55	B	15	0	0	15
56	A	15	0	0	15
56	B	15	0	0	15
57	A	15	0	0	15
57	B	15	0	0	15
58	A	15	0	0	15
58	B	15	0	0	15
59	A	15	0	0	15
59	B	15	0	0	15
60	A	15	0	0	15
60	B	15	0	0	15
61	A	15	0	0	15
61	B	15	0	0	15
62	A	15	0	0	15
62	B	15	0	0	15

Table 26
8.89 mg/L: Larval Mortality After Thinning for the 90-Day Rainbow Trout,
Oncorhynchus mykiss, Early Life-Stage Toxicity Test (Continued)

Day	Replicate	Larvae at Transfer	Dead Larvae Removed	Cumulative Dead Larvae	Live Larvae Remaining	
63	A	15	0	0	15	
63	B	15	0	0	15	
64	A	15	0	0	15	
64	B	15	0	0	15	
65	A	15	0	0	15	
65	B	15	0	0	15	
66	A	15	0	0	15	
66	B	15	0	0	15	
67	A	15	0	0	15	
67	B	15	0	0	15	
68	A	15	0	0	15	
68	B	15	0	0	15	
69	A	15	0	0	15	
69	B	15	0	0	15	
70	A	15	0	0	15	
70	B	15	0	0	15	
71	A	15	0	0	15	
71	B	15	0	0	15	
72	A	15	0	0	15	
72	B	15	0	0	15	
73	A	15	0	0	15	
73	B	15	0	0	15	
74	A	15	0	0	15	(1a)
74	B	15	0	0	15	
75	A	15	0	0	15	(1a)
75	B	15	0	0	15	
76	A	15	0	0	15	(1a)
76	B	15	0	0	15	
77	A	15	0	0	15	(1a)
77	B	15	0	0	15	
78	A	15	0	0	15	(1a)
78	B	15	0	0	15	

Table 26
8.89 mg/L: Larval Mortality After Thinning for the 90-Day Rainbow Trout,
Oncorhynchus mykiss, Early Life-Stage Toxicity Test (Continued)

Day	Replicate	Larvae at Transfer	Dead Larvae Removed	Cumulative Dead Larvae	Live Larvae Remaining
79	A	15	0	0	15 (1a,1b,1b,1b)
79	B	15	0	0	15 (1b,1b)
80	A	15	0	0	15 (1a,1b)
80	B	15	0	0	15
81	A	15	0	0	15 (1a)
81	B	15	0	0	15
82	A	15	0	0	15
82	B	15	0	0	15
83	A	15	0	0	15
83	B	15	0	0	15
84	A	15	0	0	15 (1a)
84	B	15	0	0	15
85	A	15	0	0	15 (1a)
85	B	15	0	0	15
86	A	15	0	0	15 (1a)
86	B	15	0	0	15
87	A	15	0	0	15 (1a)
87	B	15	0	0	15
88	A	15	0	0	15 (1a)
88	B	15	0	0	15
89	A	15	0	0	15 (1a)
89	B	15	0	0	15
90	A	15	0	0	15 (1a)
90	B	15	0	0	15

- a pale in color
b display of aggressive behavior

Table 27
Dilution Water Control: Fish Lengths and Weights at Test Conclusion for the 90-Day Rainbow Trout, *Oncorhynchus mykiss*, Early Life-Stage Toxicity Test

Number	Replicate	Standard Length (cm)	Wet Weight (grams)
1	A	5.6	2.50525
2	A	4.4	1.29821
3	A	5.1	1.73258
4	A	4.8	1.68578
5	A	4.6	1.42175
6	A	4.4	1.15627
7	A	4.7	1.53122
8	A	5.5	2.56945
9	A	4.7	1.54188
10	A	4.9	1.67347
11	A	5.3	2.04013
12	A	4.6	1.33401
13	A	4.8	1.50060
14	A	4.9	1.56427
1	B	5.2	2.19616
2	B	4.9	1.76121
3	B	4.6	1.48409
4	B	4.8	1.62166
5	B	4.8	1.65461
6	B	5.1	1.96772
7	B	5.5	2.65185
8	B	4.8	1.75325
9	B	4.7	1.60317
10	B	3.5	0.64408
11	B	4.8	1.64673
12	B	4.9	1.67911
13	B	4.1	0.91507
14	B	5.1	1.73033

Table 28
0.651 mg/L: Fish Lengths and Weights at Test Conclusion for the 90-Day Rainbow Trout,
Oncorhynchus mykiss, Early Life-Stage Toxicity Test

Number	Replicate	Standard Length (cm)	Wet Weight (grams)
1	A	4.7	1.65031
2	A	4.9	1.82206
3	A	4.6	1.53887
4	A	4.9	1.73448
5	A	4.8	1.63664
6	A	5.1	1.82045
7	A	5.7	2.94053
8	A	5.5	2.68676
9	A	4.4	1.17350
10	A	5.0	1.71154
11	A	4.8	1.53093
12	A	4.7	1.53887
13	A	4.8	1.52135
14	A	4.8	1.62574
1	B	4.9	1.84659
2	B	4.7	1.46995
3	B	5.5	2.60047
4	B	4.7	1.48978
5	B	5.2	1.86145
6	B	5.1	1.74391
7	B	4.9	1.50426
8	B	4.9	1.60493
9	B	5.0	1.72533
10	B	5.1	1.75725
11	B	4.7	1.41453
12	B	4.8	1.47540
13	B	4.9	1.60441
14	B	4.9	1.68552
15	B	4.9	1.65938

Table 29
1.08 mg/L: Fish Lengths and Weights at Test Conclusion for the 90-Day Rainbow Trout,
Oncorhynchus mykiss, Early Life-Stage Toxicity Test

Number	Replicate	Standard Length (cm)	Wet Weight (grams)
1	A	4.8	1.62301
2	A	5.2	2.29449
3	A	5.4	2.58264
4	A	5.0	1.75270
5	A	4.5	1.34206
6	A	4.8	1.57778
7	A	4.9	1.74969
8	A	5.1	1.75048
9	A	4.7	1.54876
10	A	4.8	1.49849
11	A	5.0	1.68845
12	A	4.8	1.55647
13	A	5.0	1.69135
14	A	5.0	1.59147
15	A	4.9	1.69665
1	B	5.1	1.84659
2	B	4.9	1.72297
3	B	4.9	1.75001
4	B	4.9	1.65018
5	B	5.0	2.01816
6	B	4.9	1.77799
7	B	5.2	1.91417
8	B	5.1	1.92946
9	B	5.6	2.57297
10	B	5.3	2.02626
11	B	4.9	1.68271
12	B	4.9	1.57688
13	B	4.9	1.54889
14	B	5.2	2.08027

Table 30
2.16 mg/L: Fish Lengths and Weights at Test Conclusion for the 90-Day Rainbow Trout,
Oncorhynchus mykiss, Early Life-Stage Toxicity Test

Number	Replicate	Standard Length (cm)	Wet Weight (grams)
1	A	5.0	1.70265
2	A	4.8	1.47242
3	A	4.6	1.44235
4	A	5.3	2.21758
5	A	5.0	1.66296
6	A	4.9	1.65316
7	A	4.6	1.60513
8	A	5.1	1.88914
9	A	5.0	1.68871
10	A	4.9	1.71284
11	A	4.9	1.68551
12	A	5.2	2.14246
13	A	4.6	1.30716
14	A	5.1	2.05367
15	A	4.4	1.21737
1	B	4.6	1.57296
2	B	4.9	1.73164
3	B	4.5	1.27899
4	B	4.7	1.49392
5	B	4.9	1.84684
6	B	5.0	1.64371
7	B	5.2	1.63336
8	B	4.6	1.45754
9	B	5.4	2.34586
10	B	5.5	2.52282
11	B	4.6	1.46046
12	B	4.8	1.57844
13	B	4.6	1.57541
14	B	5.1	1.88236
15	B	4.6	1.45194

Table 31
4.66 mg/L: Fish Lengths and Weights at Test Conclusion for the 90-Day Rainbow Trout,
Oncorhynchus mykiss, Early Life-Stage Toxicity Test

Number	Replicate	Standard Length (cm)	Wet Weight (grams)
1	A	4.8	1.70071
2	A	4.7	1.46507
3	A	4.9	1.71759
4	A	4.7	1.67281
5	A	4.4	1.17830
6	A	4.9	1.59198
7	A	5.2	2.03567
8	A	5.0	1.72793
9	A	5.5	2.54423
10	A	4.8	1.63212
11	A	4.8	1.64679
12	A	5.2	2.19754
13	A	4.7	1.59290
14	A	4.4	1.30308
15	A	4.6	1.48702
1	B	4.7	1.52371
2	B	5.8	3.01567
3	B	4.9	1.73451
4	B	4.6	1.45060
5	B	3.8	1.76098
6	B	4.7	1.50886
7	B	4.2	1.17064
8	B	4.8	1.54270
9	B	4.4	1.28983
10	B	4.6	1.43980
11	B	4.5	1.31611
12	B	4.9	1.90347
13	B	4.7	1.46469
14	B	5.1	1.89674
15	B	4.7	1.33201

Table 32
8.89 mg/L: Fish Lengths and Weights at Test Conclusion for the 90-Day Rainbow Trout,
Oncorhynchus mykiss, Early Life-Stage Toxicity Test

Number	Replicate	Standard Length (cm)	Wet Weight (grams)
1	A	4.7	1.55522
2	A	4.8	1.64880
3	A	4.8	1.51557
4	A	5.0	1.70443
5	A	4.9	1.55444
6	A	4.9	1.65174
7	A	4.8	1.53842
8	A	5.4	2.41160
9	A	4.9	1.57323
10	A	5.5	2.71202
11	A	4.8	1.65256
12	A	4.7	1.54668
13	A	4.8	1.76515
14	A	5.1	2.01093
15	A	4.6	1.55865
1	B	4.9	1.53244
2	B	4.8	1.61877
3	B	5.1	1.68852
4	B	4.8	1.56061
5	B	4.7	1.48635
6	B	5.2	2.15586
7	B	4.8	1.62254
8	B	4.9	1.82800
9	B	4.8	1.54832
10	B	4.6	1.34940
11	B	4.9	1.66576
12	B	4.9	1.65564
13	B	5.5	2.73012
14	B	4.7	1.55076
15	B	5.1	1.89430

Table 33
90-day EC₅₀, NOEC, and LOEC Values for Rainbow Trout, *Oncorhynchus mykiss*, Based on Mean, Measured Concentrations of H-28548

Parameter	90-day EC ₅₀ (mg/L)	EC ₅₀ Model	90-day NOEC (mg/L)	90-day LOEC (mg/L)	NOEC and LOEC Method
Dead Eggs at Time of Hatching	>8.89	Probit	8.89	>8.89	Cochran-Armitage
First Day of Hatching	>8.89	NA	8.89	>8.89	Tie-corrected Jonckheere
Last Day of Hatching	>8.89	OECD model 2 inverse regression	1.08	2.16	Tie-corrected Jonckheere
First Day of Swim-Up	>8.89	OECD model 2 inverse regression	8.89	>8.89	Dunn
Dead Eggs at Thinning	>8.89	Probit	8.89	>8.89	Cochran-Armitage
Larval Survival at Thinning	>8.89	Probit	8.89	>8.89	Cochran-Armitage
Larval Abnormalities at Thinning	>8.89	Probit	8.89	>8.89	Cochran-Armitage
Larval Survival at Study End	>8.89	Probit	8.89	>8.89	Cochran-Armitage
Larval Abnormalities at Study End	>8.89	Probit	8.89	>8.89	Cochran-Armitage
Larval Length at Study End	>8.89	NA	8.89	>8.89	Jonckheere
Larval Weight at Study End	>8.89	NA	8.89	>8.89	Jonckheere

FIGURES

Figure 1
Photograph of an Embryo at Approximately 22 Hours Post-Fertilization

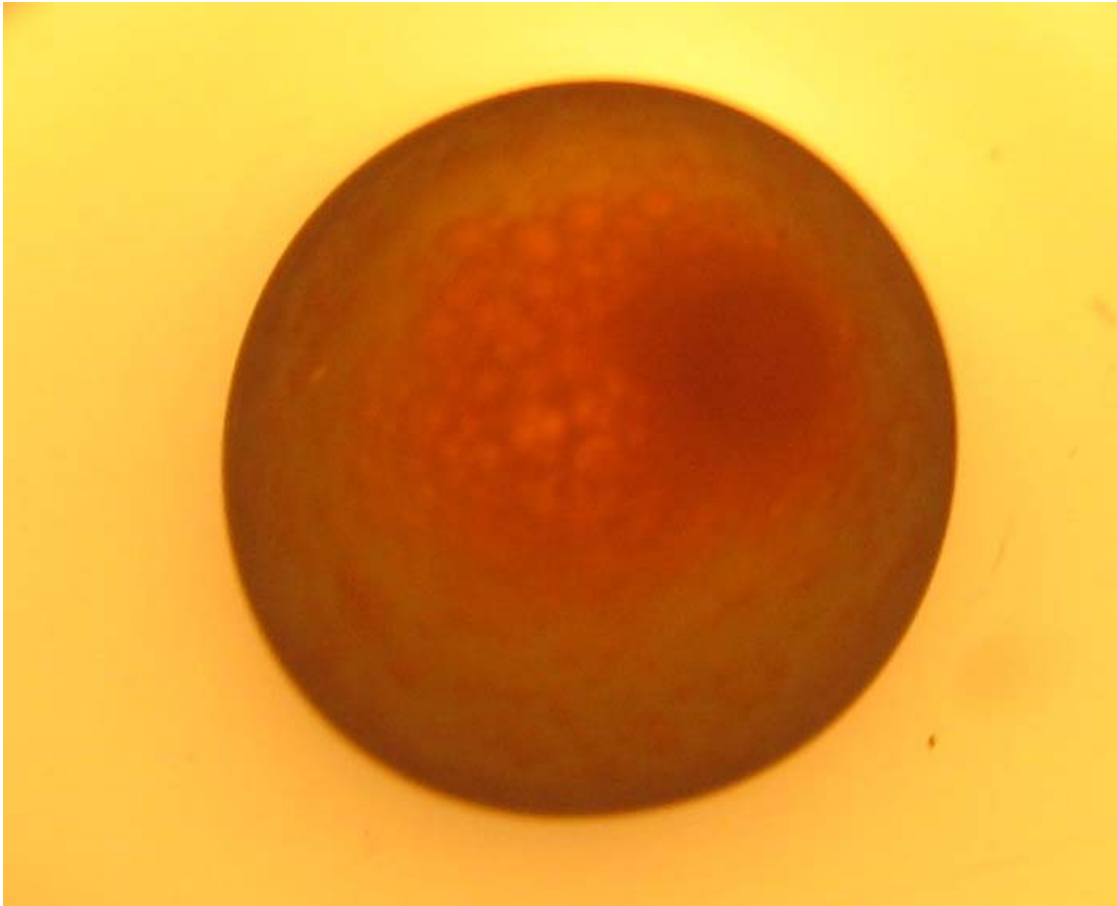


Figure 2
Representative Analytical Calibration Standard Curve for H-28548

Compound name: H-28548
Coefficient of Determination: $R^2 = 0.999806$
Calibration curve: $-1931.01 * x^2 + 6673.39 * x + -0.248072$
Response type: External Std, Area
Curve type: 2nd Order, Origin: Exclude, Weighting: $1/x$, Axis trans: None

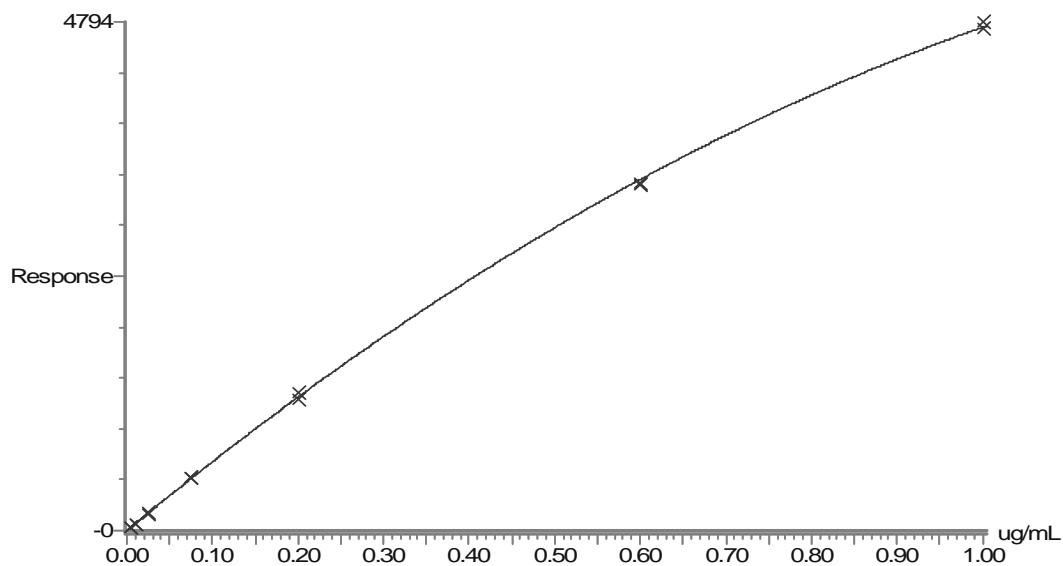
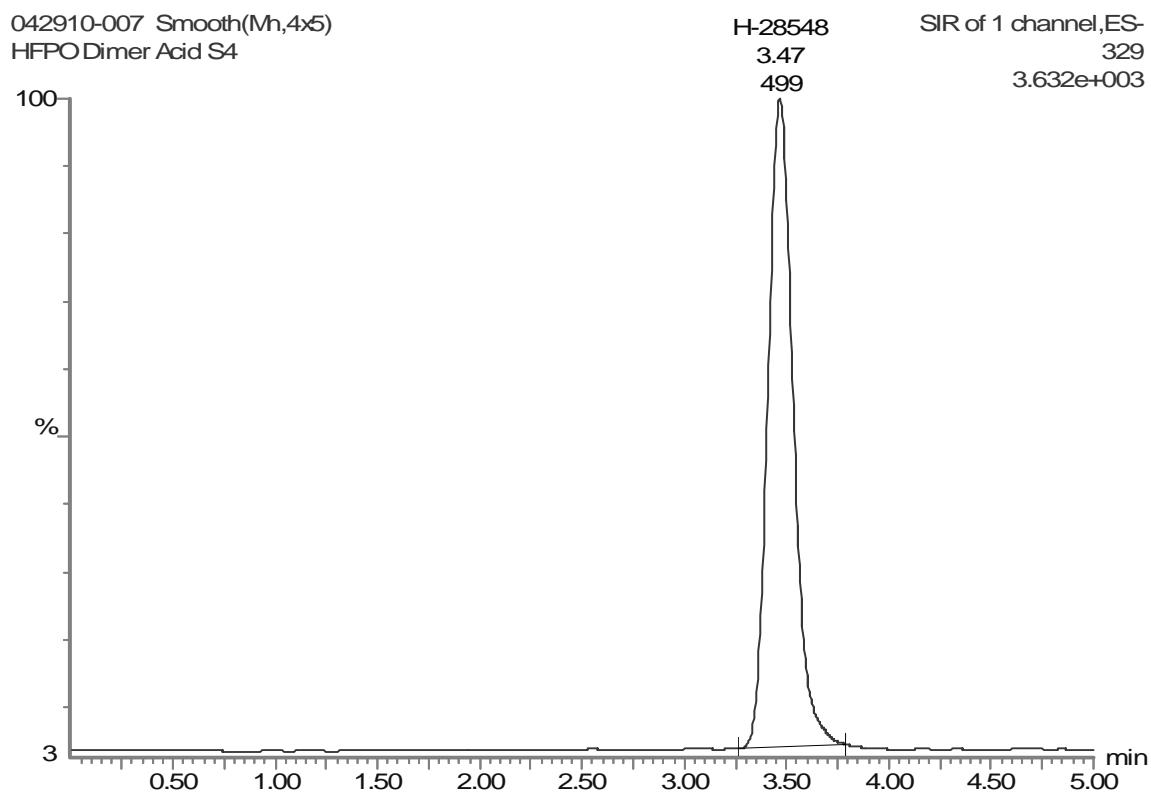
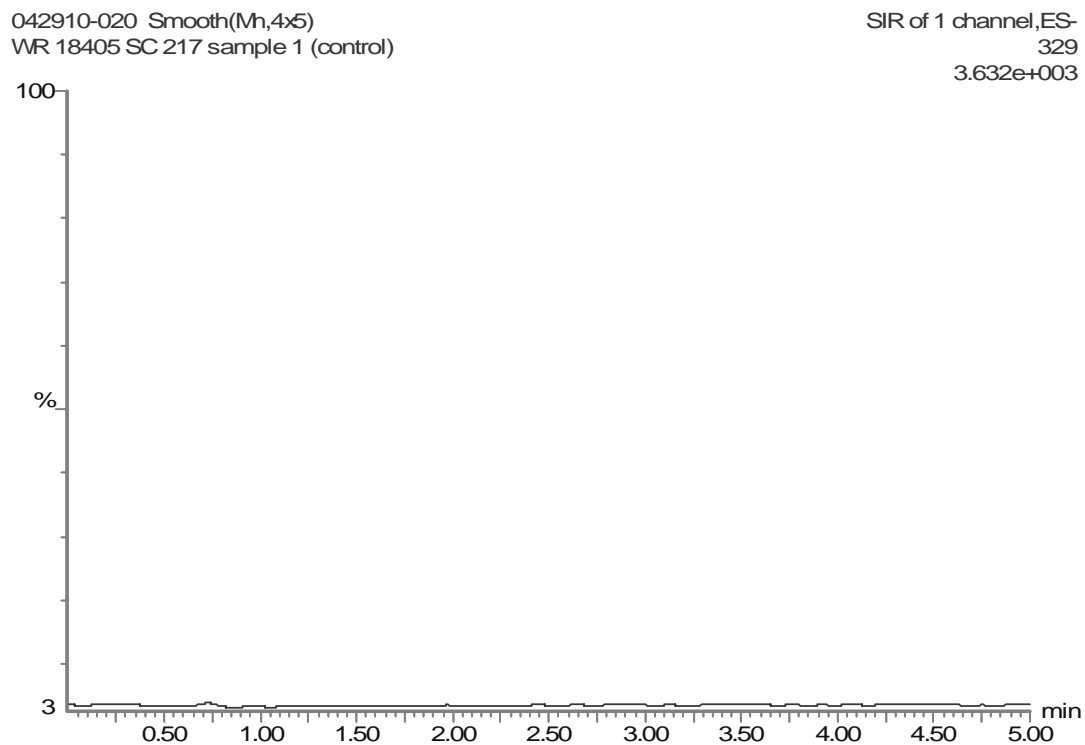


Figure 3
Representative HPLC Chromatogram of a Calibration Standard Solution



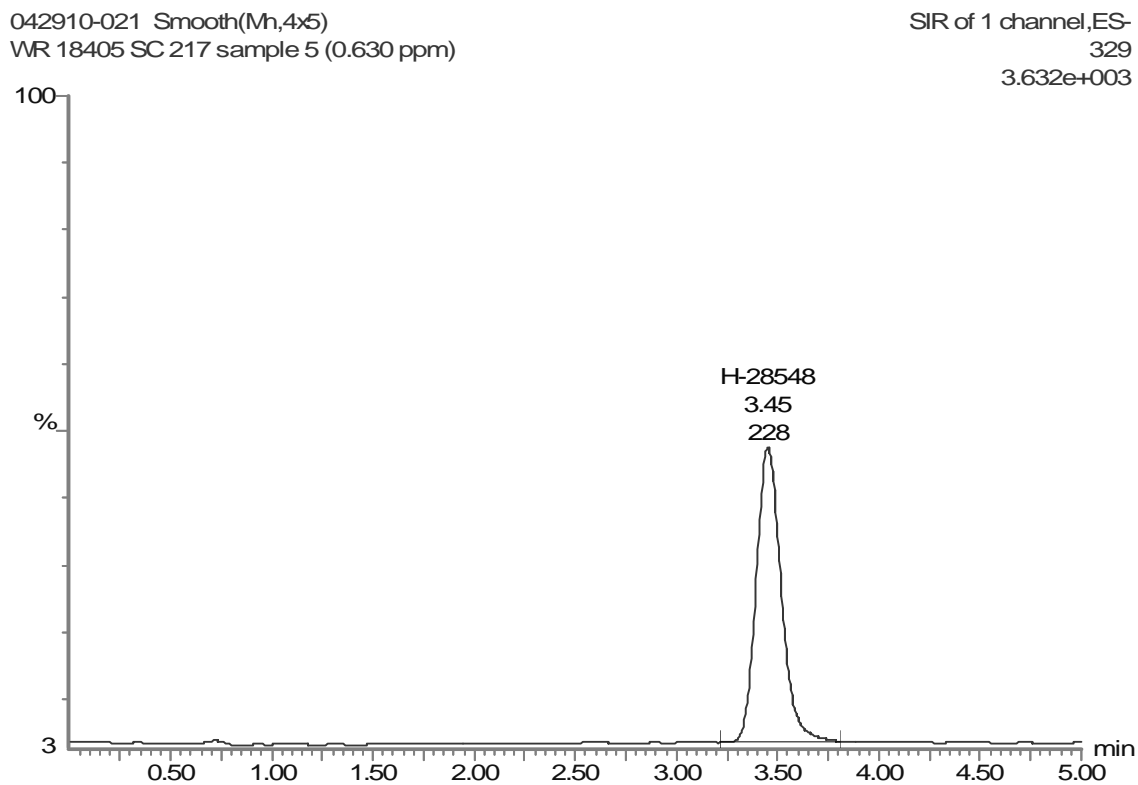
H-28548 elutes at a retention time of approximately 3.5 minutes. The calibration standard solution contains H-28548 at a concentration of 0.075 mg/L.

Figure 4
Representative HPLC Chromatogram of a HWW Control Solution



H-28548 would elute at a retention time of approximately 3.5 minutes.

Figure 5
Representative HPLC Chromatogram of a H-28548 Test Solution



H-28548 elutes at a retention time of approximately 3.5 minutes. The test solution sample contained H-28548 at a nominal concentration of 0.032 mg/L (20× dilution of the nominal 0.63 mg/L test sample).

APPENDIX

Appendix A
Certificate of Analysis




E. I. du Pont de Nemours and Company
Wilmington, DE 19898
USA

CERTIFICATE OF ANALYSIS

This Certificate of Analysis fulfills the requirement for characterization of a test substance prior to a study subject to GLP regulations. It documents the identity and content of the test substance. This work was conducted under EPA Good Laboratory Practice Standards (40 CFR 792).

Haskell Code Number	H-28548
Common Name	HFPO Dimer Acid Ammonium Salt
Purity Percent	84%
Other Components	Water – 12.7% Perfluorooctanoic acid – 150 ppm
Date of Analysis	June 13, 2008
Expiration Date	June 13, 2011
Instructions for storage	NRT&H
Reference	DuPont-25455
Analysis performed at	E. I. DuPont de Nemours and Company DuPont Haskell Laboratories Newark, Delaware USA

Approver:


Peter A. Bloxham, Ph.D.
Senior Research Chemist

24-JUN-2009
Date

Revision #1: Revised COA expiration date based on compound stability assessment. 6/23/09